U.S. PATENT APPLICATION

for

PDE5A CRYSTAL STRUCTURE AND USES

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PDE5A CRYSTAL STRUCTURE AND USES

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims the benefit of Milburn, U.S. Provisional Application 60/444,734, filed February 3, 2003 and Artis et al., U.S. Provisional Application 60/485,627, filed July 7, 2003, all of which are incorporated herein by reference in their entireties, including drawings.

BACKGROUND OF THE INVENTION

[0002] This invention relates to the field of development of ligands for phosphodiesterase 5A (PDE5A) and to the use of crystal structures of PDE5A. The information provided is intended solely to assist the understanding of the reader. None of the information provided nor references cited is admitted to be prior art to the present invention.

[0003] PDEs were first detected by Sutherland and co-workers (Rall, et al., *J. Biol. Chem.*, 232:1065-1076 (1958), Butcher, et al., *J. Biol. Chem.*, 237:1244-1250 (1962)). The superfamily of PDEs is subdivided into two major classes, class I and class II (Charbonneau, H., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds) 267-296 John Wiley & Sons, Inc., New York (1990)), which have no recognizable sequence similarity. Class I includes all known mammalian PDEs and is comprised of 11 identified families that are products of separate genes (Beavo, et al., *Mol. Pharmacol.*, 46:399-405 (1994); Conti, et al., *Endocr. Rev.*, 16:370-389 (1995); Degerman, et al., *J. Biol. Chem.*, 272:6823-6826 (1997); Houslay, M.D., *Adv. Enzyme Regul.*, 35:303-338 (1995); Bolger, G.B., *Cell Signal*, 6:851-859 (1994); Thompson, et al, *Adv. Second Messenger Phosphoprotein Res.*, 25:165-184 (1992); Underwood, et al., *J. Pharmacol. Exp. Ther.*, 270:250-259 (1994); Michaeli, et al., *J. Biol. Chem.*, 268:12925-12932 (1993); Soderling, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 95:8991-8996 (1998); Soderling, et al., *J. Biol. Chem.*, 273:15553-15558 (1998); Fisher, et al., *J. Biol. Chem.*, 273:15559-15564 (1998)). Some PDEs are highly specific for

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hydrolysis of cAMP (PDE4, PDE7, PDE8), some are highly cGMP-specific (PDE5, PDE6, PDE9), and some have mixed specificity (PDE1, PDE2, PDE3, PDE10).

[0004] All of the characterized mammalian PDEs are dimeric, but the importance of the dimeric structure for function in each of the PDEs is unknown. Each PDE has a conserved catalytic domain of ~270 amino acids with a high degree of conservation (25-30%) of amino acid sequence among PDE families, which is located carboxyl-terminal to its regulatory domain. Activators of certain PDEs appear to relieve the influence of autoinhibitory domains located within the enzyme structures (Sonnenberg, et al., *J. Biol. Chem.*, 270:30989-31000 (1995); Jin, et al., *J. Biol. Chem.*, 267:18929-18939 (1992)).

[0005] PDEs cleave the cyclic nucleotide phosphodiester bond between the phosphorus and oxygen atoms at the 3'-position with inversion of configuration at the phosphorus atom (Goldberg, et al., *J. Biol. Chem.*, 255:10344-10347 (1980); Burgers, et al., *J. Biol. Chem.*, 254:9959-9961 (1979)). This apparently results from an in-line nucleophilic attack by the OH− of ionized H_2O. It has been proposed that metals bound in the conserved metal binding motifs within PDEs facilitate the production of the attacking OH− (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994)). The kinetic properties of catalysis are consistent with a random order mechanism with respect to cyclic nucleotide and the divalent cations(s) that are required for catalysis (Srivastava, et al., *Biochem. J.*, 308:653-658 (1995)). The catalytic domains of all known mammalian PDEs contain two sequences ($HX_3 HX_n(E/D)$) arranged in tandem, each of which resembles the single Zn^{2+}-binding site of metalloendoproteases such as thermolysin (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994)). PDE5 specifically binds Zn^{2+}, and the catalytic activities of PDE4, PDE5, and PDE6 are supported by submicromolar concentrations of Zn^{2+} (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994); Percival, et al., *Biochem. Biophys. Res. Commun.*, 241:175-180 (1997)). Whether each of the Zn^{2+}-binding motifs binds Zn^{2+} independently or whether the two motifs interact to form a novel Zn^{2+}-binding site is not known. The catalytic mechanism for cleaving phosphodiester bonds of cyclic nucleotides by PDEs may be similar to that of certain proteases for cleaving the amide ester of peptides, but the presence of two Zn^{2+} motifs arranged in tandem in PDEs is unprecedented.

[0006] The group of Sutherland and Rall (Berthet, et al., *J. Biol. Chem.*, 229:351-361 (1957)), in the late 1950s, was the first to realize that at least part of the mechanism(s)

whereby caffeine enhanced the effect of glucagon, a stimulator of adenylyl cyclase, on cAMP accumulation and glycogenolysis in liver involved inhibition of cAMP PDE activity. Since that time chemists have synthesized thousands of PDE inhibitors, including the widely used 3-isobutyl-1-methylxanthine (IBMX). Many of these compounds, as well as caffeine, are non-selective and inhibit many of the PDE families. One important advance in PDE research has been the discovery/design of family-specific inhibitors such as the PDE4 inhibitor, rolipram, and the PDE5 inhibitor, sildenafil.

[0007] Precise modulation of PDE function in cells is critical for maintaining cyclic nucleotide levels within a narrow rate-limiting range of concentrations. Increases in cGMP of 2-4-fold above the basal level will usually produce a maximum physiological response. There are three general schemes by which PDEs are regulated: (*a*) regulation by substrate availability, such as by stimulation of PDE activity by mass action after elevation of cyclic nucleotide levels or by alteration in the rate of hydrolysis of one cyclic nucleotide because of competition by another, which can occur with any of the dual specificity PDEs (*e.g.* PDE1, PDE2, PDE3); (*b*) regulation by extracellular signals that alter intracellular signaling (*e.g.* phosphorylation events, Ca^{2+}, phosphatidic acid, inositol phosphates, protein-protein interactions, etc.) resulting, for example, in stimulation of PDE3 activity by insulin (Degerman, et al., *J. Biol. Chem.*, 272:6823-6826 (1997)), stimulation of PDE6 activity by photons through the transducin system (Yamazaki, et al., *J. Biol. Chem.*, 255:11619-11624 (1980)), which alters PDE6 interaction with this enzyme, or stimulation of PDE1 activity by increased interaction with Ca^{2+}/calmodulin; (*c*) feedback regulation, such as by phosphorylation of PDE1, PDE3, or PDE4 catalyzed by PKA after cAmP elevation (Conti, et al., *Endocr. Rev.*, 16:370-389 (1995); Degerman, et al., *J. Biol. Chem.*, 272:6823-6826 (1997); Gettys, et al., *J. Biol. Chem.* 262:333-339 (1987); Florio, et al, *Biochemistry*, 33:8948-8954 (1994)), by allosteric cGMP binding to PDE2 to promote breakdown of cAMP or cGMP after cGMP elevation, or by modulation of PDE protein levels, such as the desensitization that occurs by increased concentrations of PDE3 or PDE4 following chronic exposure of cells to cAMP-elevating agents (Conti, et al., *Endocr. Rev.*, 16:370-389 (1995), Sheth, et al., *Throm. Haemostasis*, 77:155-162 (1997)) or by developmentally related changes in PDE5 content. Other factors that could influence any of the three schemes outlined above are cellular compartmentalization of PDEs (Houslay, M.D., *Adv. Enzyme Regul.*, 35:303-338 (1995)) effected by covalent

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modifications such as prenylation or by specific targeting sequences in the PDE primary structure and perhaps translocation of PDEs between compartments within a cell.

[0008] Within the PDE superfamily, four (PDE2, PDE5, PDE6, and PDE10) of the 10 families contain highly cGMP-specific allosteric (non-catalytic) cGMP-binding sites in addition to a catalytic site of varying substrate specificity. Each of the monomers of these dimeric cGMP-binding PDEs contains two homologous cGMP-binding sites of ~110 amino acids arranged in tandem and located in the amino-terminal portion of the protein (Charbonneau, H., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds) 267-296 (1990); McAllister-Lucas, et al., *J. Biol. Chem.*, 270:30671-30679 (1995)). In PDE2, binding of the cGMP to these sites stimulates the hydrolysis of cAMP at the catalytic site (Beavo, et al., *Mol. Pharmacol.*, 46:399-405 (1994)). PDE2 hydrolyzed cGMP as well as cAMP, and cGMP hydrolysis is stimulated by cGMP binding at the allosteric sites in accordance with positively cooperative kinetics (Manganiello, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation, and Drug Action*, Beavo, J., and Houslay, M.D., eds, 61-85 John Wiley & Sons, Inc., New York (1990)). This could represent a negative feedback process for regulation of tissue cGMP levels (Manganiello, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation, and Drug Action*, Beavo, J., and Houslay, M.D., eds, 61-85 John Wiley & Sons, Inc., New York (1990)), which occurs in addition to the cross-talk between cyclic nucleotide pathways represented by cGMP stimulation of cAMP breakdown. Binding of cGMP to the allosteric sites of PDE6 has not been shown to affect catalysis, but this binding may modulate the interaction of PDE6 with the regulatory protein, transducin, and the inhibitory γ subunit of PDE6 (Yamazaki, et al., *Adv. Cyclic Nucleotide Protein Phosphorylation Res.*, 16:381-392 (1984)).

[0009] The first recognized cGMP-binding PDE was discovered as a cGMP-binding protein in lung tissue during a search for cyclic nucleotide-binding proteins other than cyclic nucleotide-dependent protein kinases (Lincoln, et al., *Proc. Natl. Acad. Sci U.S.A.*, 73:2559-2563 (1976)). By DEAE-cellulose chromatography, this protein appeared as a "peak 1" cGMP-binding protein that was separated from a "peak 2" cGMP-binding protein, which was shown to be PKG. The peak 1 protein possessed both cGMP-binding as well as a distinct cGMP-specific PDE catalytic activity (Francis, et al., *J. Biol. Chem.*, 255:620-626 (1980)), and it was subsequently named PDE5. Davis and Kuo (Davis, et al.,

J. Biol. Chem., 252:4078-4084 (1977)) also described a cGMP-specific PDE activity in lung tissue, and Hamet and Coquil (Hamet, et al., *J. Cyclic Nucleotide Res.*, 4:281-290 (1978)) characterized a cGMP-binding, cGMP-specific PDE in platelets.

[0010] PDE5 has been purified and cloned (Francis, et al., *J. Biol. Chem.*, 255:620-626 (1980); Francis, et al., *Methods Enzymol.*, 159:722-729 (1988); Thomas, et al., *J. Biol. Chem.*, 265:14964-14970 (1990); McAllister-Lucas, et al., *J. Biol. Chem.*, 268:22863-22873 (1993)). Two alternatively spliced variants of PDE5 have recently been identified (Yanaka, et al., *Eur. J. Biochem.*, 255:391-399 (1998); Loughney, et al., *Gene (Amst.).*, 216:137-147 (1998)). The tissue distribution of PDE5 (subunit M_r ~100,000) commonly coincides with that of PKG. This is probably not fortuitous because both PDE5 and PKG are major intracellular receptors for cGMP, and PKG is an excellent catalyst *in vitro* for phosphorylation of PDE5 (Thomas, et al., *J. Biol. Chem.*, 265:14971-14978 (1990)).

[0011] Evidence regarding the presence of conserved Zn^{2+}-binding motifs (HX_3 $HX_n(E/D)$) in PDEs and their involvement in catalysis was first demonstrated using PDE5 (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994)). Site-directed mutagenesis confirms the catalytic importance of each residue of these motifs A and B (Turko, et al., *J. Biol. Chem.*, 273:6460-6466 (1998)). Substitution of either of the invariant aspartic acid residues (Asp-714, Asp-754) further downstream in the sequence is also highly deleterious, and each of these residues may participate in the catalytic process perhaps as a catalytic base or as a coordinating ligand for a required metal. The most dramatic increases in K_m for cGMP are caused by site-directed mutagenesis of Tyr-602 and Glu-775. These two residues could form part of the cGMP-binding pocket of the catalytic site of PDE5. Because some mutations affecting k_{cat} and K_m are juxtaposed in the primary sequence, the cGMP-binding pocket and catalytic machinery are likely to involve overlapping subdomains within the catalytic domain of PDE5. All of the components required for catalytic activity of PDE5 are contained within a single monomeric catalytic domain. (Furchgott & Vanhoutte, *FASEB J.* 3:2007-2018 (1997).)

[0012] Occupation of the allosteric cGMP-binding sites of PDE5 is required for specific phosphorylation of Ser-92 by PKG or PKA, and occupation of the binding sites is also associated with an increase in the Stokes radius of the enzyme, implying that a conformational change occurs (Francis, et al., *Methods*, 14:81-92 (1998)). A direct effect

of cGMP binding to the allosteric sites on cGMP breakdown at the catalytic site has not been demonstrated, although the principle of reciprocity (binding of cGMP at the catalytic site stimulates binding at the allosteric sites) dictates that there should be an effect (Weber, G., *Adv. Protein Chem.*, 29:1-83 (1975); Francis, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds, 117-140, John Wiley & Sons, Inc., New York (1990)). The stimulatory effect of cGMP analogs specific for the catalytic site on cGMP binding to the allosteric site(s) of PDE5 suggests that interaction of cGMP with the catalytic site precedes cGMP binding to the allosteric binding site(s) (Francis, et al., *J. Biol. Chem.*, 255:620-626 (1980); Thomas, et al., *J. Biol. Chem.*, 265:14971-14978 (1990)). This implies that upon cGMP elevation in cells, cGMP breakdown at the catalytic site would increase because of mass action (increased substrate availability). This increased cGMP interaction at the catalytic site would enhance cGMP binding at the allosteric sites, thus increasing phosphorylation of the enzyme to promote further increases in cGMP breakdown. Although experimental results are consistent with such a sequence of events, this pathway has not been proven unequivocally in broken cell systems. However, rapid phosphorylation of PDE5, which is associated with increased PDE activity, occurs in intact tissues in response to stimulation by atrial natriuretic factor and may be caused by PKG action (Wyatt, et al., *Am. J. Physiol.*, 274:H448-H455 (1998)). This process could represent negative-feedback regulation of cGMP levels in cells. PKA can also phosphorylate PDE5 *in vitro*, albeit at about 10% the rate at which PKG catalyzes this reaction; whether or not this occurs *in vivo* is uncertain because the concomitant elevation of cGMP and cAMP would be required to expose Ser-92 and activate PKA, respectively. Burns *et al.* (Burns, et al., *Biochem. J.*, 283:487-491 (1992)) have reported that a partially purified PDE5 from guinea pig lung is activated when phosphorylated by PKA. PDE5 may also be regulated by other low molecular weight factors, and these could alter the effects of phosphorylation (Lochhead, et al., *J. Biol. Chem.*, 272:18397-18403 (1997)). As is the case for PDE4, PDE5 may also be subject to long term regulation through changes in enzyme concentration in some cell types (Sanchez, et al., *Pediatr. Res.*, 43:163-168 (1998); Kotera, et al., *Eur. J. Biochem.*, 249:434-442 (1997); Bakre, et al., *FEBS Lett.*, 408:345-349 (1997)).

[0013]   The K_D of PDE5 for binding cGMP in the allosteric sites is ~0.2 μM (Thomas, et al., *J. Biol. Chem.*, 265:14964-14970 (1990)). The presence of two kinetically distinct allosteric cGMP-binding sites in PDE5 was first suggested by the curvilinear pattern of

cGMP dissociation from the enzyme. Studies using site-directed mutagenesis confirm the presence of two sites and indicate that the binding of cGMP to each allosteric site could involve a NK$(X)_n$D motif (McAllister-Lucas, et al., *J. Biol. Chem.*, 270:30671-30679 (1995); Turko, et al., *J. Biol. Chem.*, 271:22240-22244 (1996)), which resembles that used by G proteins for binding GTP (Pai, et al, *Nature*, 341:209-214 (1989)). The conserved sequence of the allosteric cyclic nucleotide-binding sites in PDE2, PDE5, PDE6, and PDE10 is evolutionarily distinct from that of the family containing PKG, PKA, and cation channels (McAllister-Lucas, et al., *J. Biol. Chem.*, 268:22863-22873 (1993)), indicating that the allosteric cGMP-binding sites of these PDEs represent a newly recognized class of cyclic nucleotide receptors. Another class may be represented by the catalytic sites of PDEs, the sequences of which contain a binding pocket for cyclic nucleotides in the catalytic domain in order to optimize the catalytic process. In PDE5, classical PDE inhibitors and selected cyclic nucleotide analogs compete with cGMP at the catalytic site but do not interact with the cGMP-binding allosteric sites (Francis, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds, 117-140, John Wiley & Sons, Inc., New York (1990)). The order of potency of some common PDE inhibitors for PDE5 is sildenafil > zaprinast > dipyridamole > IBMX > cilostamide > theophylline > caffeine > rolipram (Fig. 3) (Thomas, et al., *J. Biol. Chem.*, 265:14964-14970 (1990); Ballard, et al., *J. Urol.*, 159:2164-2171 (1998)). Many cyclic nucleotide analogs are also inhibitors of PDE5 (Francis, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds, 117-140, John Wiley & Sons, Inc., New York (1990)), which is to be expected based on the structural similarity of these compounds with cGMP. Some IBMX analogs modified at the 8-position, such as 8-(2-chlorobenzyl)-IBMX, are more potent inhibitors than are any of the cyclic nucleotide analogs (Sekhar, et al., *Phosphodiesterase Inhibitors*, Schudt, C., Dent, G., and Rabe, K.F., eds, 135-146, Academic Press, New York (1996)). Even though the IBMX analogs are generally better PDE5 inhibitors than are cyclic nucleotide analogs, many of the latter are more potent for relaxing intact vascular smooth muscle.

[0014] Because the PDE inhibitors show competitive kinetics with respect to cGMP in the catalytic site of PDE5, they would be expected to form molecular contacts like those formed by cGMP. However, results of mutagenesis of PDE5 indicate that, although both zaprinast, a potent PDE5 inhibitor, and cGMP appear to make contact with several of the

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same amino acids in the catalytic domain, some of the residues that are important for interaction with zaprinast, *e.g.* Asp-754 and Gly-780, are not critical for interaction with cGMP (Turko, et al., *J. Biol. Chem.*, 273:6460-6466 (1998)). As noted above, Asp-754 is crucial for efficient catalysis, which is suggestive that inhibition by zaprinast could be due in part to interference with an important function of Asp-754.

[0015] The PDE5 subfamily has only one member: PDE5A (Corbin and Francis, "Cyclic GMP Phosphodiesterase-5: Target of Sildenafil," *The Journal of Biological Chemistry*, 274(20):13729-13732 (1999)). PDE5 possesses a preference for cGMP over cAMP as a substrate. PDE5 is expressed in smooth muscle tissue (Table 1), importantly in the corpus cavernosum. This enzyme possesses two GAF domains in the N-terminal regulatory region. These GAF domains act in concert to bind cGMP and mediate dimerization and activation PDE activity. A recent crystal structure of the PDE2 GAF domain suggests possible mechanisms by which the GAF domains bind cGMP and mediate dimerization (Martinez et al., *Proc Natl Acad Sci USA* 99:13260-13265 (2002)). PDE5 has attracted considerable attention as a therapeutic target due to the tremendous commercial success of Viagra (Pfizer) (Rotella, 2002, Phosphodiesterase 5 inhibitors: Current status and potential applications, *Nature Reviews* 1:674-682). In addition to Viagra (sildenafil), two other drugs are quite far along in the approval process, namely vardenafil (Bayer) and tadalafil (Lilly/ICOS). One apparent drawback to these compounds is some cross-reactivity with the closely related PDE families PDE6 and PDE11 (Gresser and Gleiter, *Eur J Med Res* 7:435-446 (2002)). The availability of PDE5 structural information may enable the discovery of PDE5 inhibitors with improved selectivity versus PDE6 and PDE11. The crystal structure of PDE5 has not been reported in the literature.

SUMMARY OF THE INVENTION

[0016] The present invention concerns structural information about PDE5A, crystals of PDE5A with and without binding compounds, and the use of the PDE5A crystals and structural information about the PDE5A to develop PDE5A ligands, which can be developed from new chemical classes, or can be developed from previously known PDE5A ligands.

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[0017] Thus, in a first aspect, the invention concerns a method for developing ligands binding to a PDE5A, where the method includes identifying as molecular scaffolds one or more compounds that bind to a binding site of PDE5A; determining the orientation of at least one molecular scaffold in co-crystals with PDE5A; identifying chemical structures of one or more of the molecular scaffolds, that, when modified, alter the binding affinity or binding specificity or both between the molecular scaffold and the PDE5A; and synthesizing a ligand in which one or more of the chemical structures of the molecular scaffold is modified to provide a ligand that binds to the PDE5A with altered binding affinity or binding specificity or both.

[0018] The terms "PDE5A phosphodiesterase" and "PDE5A" mean an enzymatically active phosphodiesterase that contains a portion with greater than 90% amino acid sequence identity to amino acid residues 531-875 of native PDE5A as shown in Table 4, for a maximal alignment over an equal length segment; or that contains a portion with greater than 90% amino acid sequence identity to at least 200 contiguous amino acids from amino acid residues 531-875 of native PDE5A or the amino acid sequence provided in Table 2 that retains binding to natural PDE5A ligand cGMP. Preferably the sequence identity is at least 95, 97, 98, 99, or even 100%. Preferably the specified level of sequence identity is over a sequence at least 300 contiguous amino acid residues.

[0019] The term "PDE5A phosphodiesterase domain" refers to a reduced length PDE5A (*i.e.*, shorter than a full-length PDE5A by at least 100 amino acids that includes the phosphodiesterase catalytic region in PDE5A. Highly preferably for use in this invention, the phosphodiesterase domain retains phosphodiesterase activity, preferably at least 50% the level of phosphodiesterase activity as compared to the native PDE5A, more preferably at least 60, 70, 80, 90, or 100% of the native activity.

[0020] As used herein, the terms "ligand" and "modulator" are used equivalently to refer to a compound that modulates the activity of a target biomolecule, *e.g.*, an enzyme such as a kinase or phosphodiesterase. Generally a ligand or modulator will be a small molecule, where "small molecule refers to a compound with a molecular weight of 1500 daltons or less, or preferably 1000 daltons or less, 800 daltons or less, or 600 daltons or less. Thus, an "improved ligand" is one that possesses better pharmacological and/or pharmacokinetic properties than a reference compound, where "better" can be defined by a

person for a particular biological system or therapeutic use. In terms of the development of ligands from scaffolds, a ligand is a derivative of a scaffold.

[0021] In the context of binding compounds, molecular scaffolds, and ligands, the term "derivative" or "derivative compound" refers to a compound having a chemical structure that contains a common core chemical structure as a parent or reference compound, but differs by having at least one structural difference, *e.g.*, by having one or more substituents added and/or removed and/or substituted, and/or by having one or more atoms substituted with different atoms. Unless clearly indicated to the contrary, the term "derivative" does not mean that the derivative is synthesized using the parent compound as a starting material or as an intermediate, although in some cases, the derivative may be synthesized from the parent.

[0022] Thus, the term "parent compound" refers to a reference compound for another compound, having structural features continued in the derivative compound. Often but not always, a parent compound has a simpler chemical structure than the derivative.

[0023] By "chemical structure" or "chemical substructure" is meant any definable atom or group of atoms that constitute a part of a molecule. Normally, chemical substructures of a scaffold or ligand can have a role in binding of the scaffold or ligand to a target molecule, or can influence the three-dimensional shape, electrostatic charge, and/or conformational properties of the scaffold or ligand.

[0024] The term "binds" in connection with the interaction between a target and a potential binding compound indicates that the potential binding compound associates with the target to a statistically significant degree as compared to association with proteins generally (*i.e.*, non-specific binding). Thus, the term "binding compound" refers to a compound that has a statistically significant association with a target molecule. Preferably a binding compound interacts with a specified target with a dissociation constant (k_d) of 1 mM or less. A binding compound can bind with "low affinity", "very low affinity", "extremely low affinity", "moderate affinity", "moderately high affinity", or "high affinity" as described herein.

[0025] In the context of compounds binding to a target, the term "greater affinity" indicates that the compound binds more tightly than a reference compound, or than the

same compound in a reference condition, *i.e.*, with a lower dissociation constant. In particular embodiments, the greater affinity is at least 2, 3, 4, 5, 8, 10, 50, 100, 200, 400, 500, 1000, or 10,000-fold greater affinity.

[0026] Also in the context of compounds binding to a biomolecular target, the term "greater specificity" indicates that a compound binds to a specified target to a greater extent than to another biomolecule or biomolecules that may be present under relevant binding conditions, where binding to such other biomolecules produces a different biological activity than binding to the specified target. Typically, the specificity is with reference to a limited set of other biomolecules, *e.g.*, in the case of PDE5A, other phosphodiesterases (*e.g.*, PDE1, PDE6, and/or PDE11) or even other type of enzymes. In particular embodiments, the greater specificity is at least 2, 3, 4, 5, 8, 10, 50, 100, 200, 400, 500, or 1000-fold greater specificity.

[0027] As used in connection with binding of a compound with PDE5A, the term "interact" indicates that the distance from a bound compound to a particular amino acid residue will be 5.0 angstroms or less. In particular embodiments, the distance from the compound to the particular amino acid residue is 4.5 angstroms or less, 4.0 angstroms or less, or 3.5 angstroms or less. Such distances can be determined, for example, using co-crystallography, or estimated using computer fitting of a compound in a PDE5A active site.

[0028] For reference to particular amino acid residues in PDE5A, polypeptide residue number is defined by the numbering provided in Yanaka et al., 1998, *Eur. J. Biochem.* 255:391-399.

[0029] In a related aspect, the invention provides a method for developing ligands specific for PDE5A, where the method involves determining whether a derivative of a compound that binds to a plurality of phosphodiesterases (e.g., a molecular scaffold) has greater specificity for the PDE5A phosphodiesterase than the parent compound with respect to other phosphodiesterases.

[0030] As used herein in connection with binding compounds or ligands, the term "specific for PDE5A phosphodiesterase", "specific for PDE5A" and terms of like import mean that a particular compound binds to PDE5A to a statistically greater extent than to

other phosphodiesterases that may be present in a particular organism. Also, where biological activity other than binding is indicated, the term "specific for PDE5A" indicates that a particular compound has greater biological activity associated with binding PDE5A than to other phosphodiesterases. Preferably, the specificity is also with respect to other biomolecules (not limited to phosphodiesterases) that may be present from an organism.

[0031] In another aspect, the invention provides a method for obtaining improved ligands binding to PDE5A, where the method involves identifying a compound that binds to PDE5A, determining whether that compound interacts with one or more conserved PDE5A active site residues, and determining whether a derivative of that compound binds to the PDE5A with greater affinity or greater specificity or both than the parent binding compound. Binding with greater affinity or greater specificity or both than the parent compound indicates that the derivative is an improved ligand. This process can also be carried out in successive rounds of selection and derivatization and/or with multiple parent compounds to provide a compound or compounds with improved ligand characteristics. Likewise, the derivative compounds can be tested and selected to give high selectivity for PDE5A, or to give cross-reactivity to a particular set of targets, for example to a subset of phosphodiesterases that includes PDE5A. In particular embodiments, known PDE5A inhibitors can be used, and derivatives with greater affinity and/or greater specificity can be developed, preferably using PDE5A structure information; greater specificity for PDE5A relative to PDE1, PDE6, and/or PDE11 is developed.

[0032] By "molecular scaffold" or "scaffold" is meant a simple target binding molecule to which one or more additional chemical moieties can be covalently attached, modified, or eliminated to form a plurality of molecules with common structural elements. The moieties can include, but are not limited to, a halogen atom, a hydroxyl group, a methyl group, a nitro group, a carboxyl group, or any other type of molecular group including, but not limited to, those recited in this application. Molecular scaffolds bind to at least one target molecule, preferably to a plurality of molecules in a protein family, and the target molecule can preferably be a enzyme, receptor, or other protein. Preferred characteristics of a scaffold can include binding at a target molecule binding site such that one or more substituents on the scaffold are situated in binding pockets in the target molecule binding site; having chemically tractable structures that can be chemically modified, particularly by synthetic reactions, so that a combinatorial library can be easily constructed; having

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chemical positions where moieties can be attached that do not interfere with binding of the scaffold to a protein binding site, such that the scaffold or library members can be modified to form ligands, to achieve additional desirable characteristics, *e.g.*, enabling the ligand to be actively transported into cells and/or to specific organs, or enabling the ligand to be attached to a chromatography column for additional analysis. Thus, a molecular scaffold is an identified target binding molecule prior to modification to improve binding affinity and/or specificity, or other pharmacalogic properties.

[0033] The term "scaffold core" refers to the core structure of a molecular scaffold onto which various substituents can be attached. Thus, for a number of scaffold molecules of a particular chemical class, the scaffold core is common to all the scaffold molecules. In many cases, the scaffold core will consist of or include one or more ring structures.

[0034] By "binding site" is meant an area of a target molecule to which a ligand can bind non-covalently. Binding sites embody particular shapes and often contain multiple binding pockets present within the binding site. The particular shapes are often conserved within a class of molecules, such as a molecular family. Binding sites within a class also can contain conserved structures such as, for example, chemical moieties, the presence of a binding pocket, and/or an electrostatic charge at the binding site or some portion of the binding site, all of which can influence the shape of the binding site.

[0035] By "binding pocket" is meant a specific volume within a binding site. A binding pocket can often be a particular shape, indentation, or cavity in the binding site. Binding pockets can contain particular chemical groups or structures that are important in the non-covalent binding of another molecule such as, for example, groups that contribute to ionic, hydrogen bonding, or van der Waals interactions between the molecules.

[0036] By "orientation", in reference to a binding compound bound to a target molecule is meant the spatial relationship of the binding compound (which can be defined by reference to at least some of its consitituent atoms) to the binding pocket and/or atoms of the target molecule at least partially defining the binding pocket.

[0037] In the context of target molecules in this invention, the term "crystal" refers to a regular assemblage of a target molecule of a type suitable for X-ray crystallography. That is, the assemblage produces an X-ray diffraction pattern when illuminated with a beam of

X-rays. Thus, a crystal is distinguished from an aggolmeration or other complex of target molecule that does not give a diffraction pattern.

[0038] By "co-crystal" is meant a complex of the compound, molecular scaffold, or ligand bound non-covalently to the target molecule and present in a crystal form appropriate for analysis by X-ray or protein crystallography. In preferred embodiments the target molecule-ligand complex can be a protein-ligand complex.

[0039] The phrase "alter the binding affinity or binding specificity" refers to changing the binding constant of a first compound for another, or changing the level of binding of a first compound for a second compound as compared to the level of binding of the first compound for third compounds, respectively. For example, the binding specificity of a compound for a particular protein is increased if the relative level of binding to that particular protein is increased as compared to binding of the compound to unrelated proteins.

[0040] As used herein in connection with test compounds, binding compounds, and modulators (ligands), the term "synthesizing" and like terms means chemical synthesis from one or more precursor materials.

[0041] The phrase "chemical structure of the molecular scaffold is modified" means that a derivative molecule has a chemical structure that differs from that of the molecular scaffold but still contains common core chemical structural features. The phrase does not necessarily mean that the molecular scaffold is used as a precursor in the synthesis of the derivative.

[0042] By "assaying" is meant the creation of experimental conditions and the gathering of data regarding a particular result of the experimental conditions. For example, enzymes can be assayed based on their ability to act upon a detectable substrate. A compound or ligand can be assayed based on its ability to bind to a particular target molecule or molecules.

[0043] By a "set" of compounds is meant a collection of compounds. The compounds may or may not be structurally related.

[0044] In another aspect, structural information about PDE5A can also be used to assist in determining a struture for another phosphodiesterase, *e.g.*, a PDE2, by creating a homology model from an electronic representation of a PDE5A structure.

[0045] Typically creating such a homology model involves identifying conserved amino acid residues between PDE5A and the other phosphodiesterase of interest; transferring the atomic coordinates of a plurality of conserved amino acids in the PDE5A structure to the corresponding amino acids of the other phosphodiesterase to provide a rough structure of that phosphodiesterase; and constructing structures representing the remainder of the other phosphodiesterase using electronic representations of the structures of the remaining amino acid residues in the other phosphodiesterase. In particular, coordinates from Table 1 for conserved residues can be used. Conserved residues in a binding site can be used.

[0046] To assist in developing other portions of the phosphodiesterase structure, the homology model can also utilize, or be fitted with, low resolution x-ray diffraction data from one or more crystals of the phosphodiesterase, *e.g.*, to assist in linking conserved residues and/or to better specify coordinates for terminal portions of a polypeptide.

[0047] The PDE5A structural information used can be for a variety of different PDE5A variants, including full-length wild type, naturally-occurring variants (*e.g.*, allelic variants and splice variants), truncated variants of wild type or naturally-occuring variants, and mutants of full-length or truncated wild-type or naturally-occurring variants (that can be mutated at one or more sites). For example, in order to provide a PDE5A structure closer to a variety of other phosphodiesterase structures, a mutated PDE5A that includes a mutation to a conserved residue in a binding site can be used.

[0048] In another aspect, the invention provides a crystalline form of PDE5A, which may be a reduced length PDE5A such as a PDE5A phosphodiesterase domain, *e.g.*, having atomic coordinates as described in Table 1. The crystalline form can contain one or more heavy metal atoms, for example, atoms useful for X-ray crystallography. The crystalline form can also include a binding compound in a co-crystal, *e.g.*, a binding compound that interacts with one more more conserved PDE5A active site residues, or any two, any three, any four, any five, any six of those residues, and can, for example, be a known PDE5A inhibitor. PDE5A crystals can be in various environments, *e.g.*, in a crystallography plate, mounted for X-ray crystallography, and/or in an X-ray beam. The PDE5A may be of

various forms, *e.g.*, a wild-type, variant, truncated, and/or mutated form as described herein.

[0049]   The invention further concerns co-crystals of PDE5A, which may be a reduced length PDE5A, *e.g.*, a PDE5A phosphodiesterase domain, and a PDE5A binding compound. Advantageously, such co-crystals are of sufficient size and quality to allow structural determination of PDE5A to at least 3 Angstroms, 2.5 Angstroms, 2.0 Angstroms, or 1.8 Angstroms. The co-crystals can, for example, be in a crystallography plate, be mounted for X-ray crystallography and/or in an X-ray beam. Such co-crystals are beneficial, for example, for obtaining structural information concerning interaction between PDE5A and binding compounds.

[0050]   PDE5A binding compounds can include compounds that interact with at least one of conserved PDE5A active site residues, or any 2, 3, 4, 5, or 6 of those residues. Exemplary compounds that bind to PDE5A include compounds described in references cited herein.

[0051]   Likewise, in additional aspects, methods for obtaining PDE5A crystals and co-crystals are provided. In one aspect is provided a method for obtaining a crystal of PDE5A phosphodiesterase domain, by subjecting PDE5A phosphodiesterase domain protein at 5-20 mg/ml, preferably 8-12 mg/ml, to crystallization condition substantially equivalent to: 10% (w/v) PEG3000, 100 mM phosphate-citrate (pH 4.3), 200 mM NaCl, 1mM DTT, 1mM Sp-cAMP. In general, the PDE5A will be in a solution containing the protein and suitable buffer.

[0052]   Crystallization conditions can be initially identified using a screening kit, such as a Hampton Research (Riverside, CA) screening kit 1. Conditions resulting in crystals can be selected and crystallization conditions optimized based on the demonstrated crystallization conditions. To assist in subsequent crystallography, the PDE5A can be seleno-methionine labeled. Also, as indicated above, the PDE5A may be any of various forms, *e.g.*, truncated to provide a PDE5A phosphodiesterase domain, which can be selected to be of various lengths.

[0053]   A related aspect provides a method for obtaining co-crystals of PDE5A, which can be a reduced length PDE5A, with a binding compound, by subjecting PDE5A protein

at 5-20 mg/ml to crystallization conditions substantially equivalent to10% (w/v) PEG3000, 100 mM phosphate-citrate (pH 4.3), 200 mM NaCl, 1mM DTT, 1mM Sp-cAMP, in the presence of binding compound, for a time sufficient for cystal development. The binding compound may be added at various concentrations depending on the nature of the comound, e.g., final concentration of 0.5 to 1.0 mM. In many cases, the binding compound will be in an organic solvent such as demethyl sulfoxide solution (DMSO). While not preferred, binding compound can also be soaked into a PDE5A crystal, e.g., using conventional techniques.

[0054] In another aspect, provision of compounds active on PDE5A also provides a method for modulating PDE5A activity by contacting PDE5A with a compound that binds to PDE5A and interacts with one more conserved PDE5A active site residues, where the compound has been identified using a PDE5A crystal structure. The compound is preferably provided at a level sufficient to modulate the activity of PDE5A by at least 10%, more preferably at least 20%, 30%, 40%, or 50%. In many embodiments, the compound will be at a concentration of about 1 μM, 100 μM, or 1 mM, or in a range of 1-100 nM, 100-500 nM, 500-1000 nM, 1-100 μM, 100-500 μM, or 500-1000 μM.

[0055] As used herein, the term "modulating" or "modulate" refers to an effect of altering a biological activity, especially a biological activity associated with a particular biomolecule such as PDE5A. For example, an agonist or antagonist of a particular biomolecule modulates the activity of that biomolecule, e.g., an enzyme.

[0056] The term "PDE5A activity" refers to a biological activity of PDE5A, particularly including phosphodiesterase activity.

[0057] In the context of the use, testing, or screening of compounds that are or may be modulators, the term "contacting" means that the compound(s) are caused to be in sufficient proximity to a particular molecule, complex, cell, tissue, organism, or other specified material that potential binding interactions and/or chemical reaction between the compound and other specified material can occur.

[0058] In a related aspect, the invention provides a method for treating a patient suffering from a disease or condition characterized by abnormal PDE5A

phosphodiesterase activity, where the method involves administering to the patient a compound identified by fitting to a PDE5A crystal structure.

[0059] Specific diseases or disorders which might be treated or prevented include those described in the Detailed Description herein, and in the references cited therein.

[0060] As crystals of PDE5A have been developed and analyzed, another aspect concerns an electronic representation of PDE5A (which may be a reduced length PDE5A), for example, an electronic representation containing atomic coordinate representations corresponding to the coordinates listed for PDE5A in Table 1, or a schematic representation such as one showing secondary structure and/or chain folding, and may also show conserved active site residues. The PDE5A may be wild type, an allelic variant, a mutant form, or a modifed form, *e.g.*, as described herein.

[0061] The electronic representation can also be modified by replacing electronic representations of particular residues with electronic representations of other residues. Thus, for example, an electronic representation containing atomic coordinate representations corresponding to the coordinates for PDE5A listed in Table 1 can be modified by the replacement of coordinates for a particular conserved residue in a binding site by a different amino acid. Likewise, a PDE5A representation can be modified by the respective substitutions, insertions, and/or deletions of amino acid residues to provide a representation of a structure for PDE6 or PDE11. Following a modification or modifications, the representation of the overall structure can be adjusted to allow for the known interactions that would be affected by the modification or modifications. In most cases, a modification involving more than one residue will be performed in an iterative manner.

[0062] In addition, an electronic representation of a PDE5A binding compound or a test compound in the binding site can be included, *e.g.*, a non-hydrolyzable cGMP analog.

[0063] Likewise, in a related aspect, the invention concerns an electronic representation of a portion of PDE5A, a binding site (which can be an active site) or phosphodiesterase domain, for example, residues 531-875 or other phosphodiesterase domain described herein, such as the amino acid sequence provided in Table 2. A binding site or phosphodiesterase domain can be represented in various ways, *e.g.*, as representations of

atomic coordinates of residues around the binding site and/or as a binding site surface contour, and can include representations of the binding character of particular residues at the binding site, e.g., conserved residues. As for electronic representations of PDE5A, a binding compound or test compound may be present in the binding site; the binding site may be of a wild type, variant, mutant form, or modified form of PDE5A.

[0064] In yet another aspect, the structural information of PDE5A can be used in a homology model (based on PDE5A) for another phosphodiesterase (such as PDE6 or PDE11), thus providing an electronic representation of a PDE5A based homology model for a phosphodiesterase. For example, the homology model can utilize atomic coordinates from Table 1 for conserved amino acid residues. In particular embodiments; atomic coordinates for a wild type, variant, modified form, or mutated form of PDE5A can be used, including, for example, wild type, variants, modified forms, and mutant forms as described herein. In particular, PDE5A structure provides a very close homology model for PDE6 and PDE11. Thus, in particular embodiments the invention provides PDE5A-based homology models of PDE6 and PDE11.

[0065] In still another aspect, the invention provides an electronic representation of a modified PDE5A crystal structure, that includes an electronic representation of the atomic coordinates of a modified PDE5A. In an exemplary embodiment, atomic coordinates of Table 1 can be modified by the replacement of atomic coordinates for a conserved residue with atomic coordinates for a different amino acid. Modifications can include substitutions, deletions (e.g., C-terminal and/or N-terminal delections), insertions (internal, C-terminal, and/or N-terminal) and/or side chain modifications.

[0066] In another aspect, the PDE5A structural information provides a method for developing useful biological agents based on PDE5A, by analyzing a PDE5A structure to identify at least one sub-structure for forming the biological agent. Such sub-structures can include epitopes for antibody formation, and the method includes developing antibodies against the epitopes, e.g., by injecting an epitope presenting composition in a mammal such as a rabbit, guinea pig, pig, goat, or horse. The sub-structure can also include a mutation site at which mutation is expected to or is known to alter the activity of the PDE5A, and the method includes creating a mutation at that site. Still further, the sub-structure can include an attachment point for attaching a separate moiety, for example, a

peptide, a polypeptide, a solid phase material (*e.g.*, beads, gels, chromatographic media, slides, chips, plates, and well surfaces), a linker, and a label (*e.g.*, a direct label such as a fluorophore or an indirect label, such as biotin or other member of a specific binding pair). The method can include attaching the separate moiety.

[0067]   In another aspect, the invention provides a method for identifying potential PDE5A, binding compounds by fitting at least one electronic representation of a compound in an electronic representation of a PDE5A binding site. The representation of the binding site may be part of an electronic representation of a larger portion(s) or all of a PDE5A molecule or may be a representation of only the binding site or active site. The electronic representation may be as described above or otherwise described herein. For example, the compound may be a molecular scaffold, a derivative of a molecular scaffold, or a compound that is structurally similar to such molecular scaffold or derivative thereof.

[0068]   In particular embodiments, the method involves fitting a computer representation of a compound from a computer database with a computer representation of the active site of PDE5A, and involves removing a computer representation of a compound complexed with the PDE5A molecule and identifying compounds that best fit the active site based on favorable geometric fit and energetically favorable complementary interactions as potential binding compounds. In particular embodiments, the compound is a known PDE5A inhibitor, *e.g.*, as described in a reference cited herein, or a derivative thereof.

[0069]   In other embodiments, the method involves modifying a computer representation of a compound complexed with a PDE5A molecule, by the deletion or addition or both of one or more chemical groups; fitting a computer representation of a compound from a computer database with a computer representation of the active site of the PDE5A molecule; and identifying compounds that best fit the active site based on favorable geometric fit and energetically favorable complementary interactions as potential binding compounds.

[0070]   In still other embodiments, the method involves removing a computer representation of a compound complexed with PDE5A, and searching a database for compounds having structural similarity to the complexed compound using a compound searching computer program or replacing portions of the complexed compound with similar chemical structures using a compound construction computer program.

[0071]   Fitting a compound can include determining whether a compound will interact with one or more conserved PDE5A active site residues. Compounds selected for fitting or that are complexed with PDE5A can, for example, be a known PDE5A inhibitor compound.

[0072]   In another aspect, the invention concerns a method for attaching a PDE5A binding compound to an attachment component, as well as a method for indentifying attachment sites on a PDE5A binding compound. The method involves identifying energetically allowed sites for attachment of an attachment component for the binding compound bound to a binding site of PDE5A; and attaching the compound or a derivative thereof to the attachment component at the energetically allowed site.

[0073]   Attachment components can include, for example, linkers (including traceless linkers) for attachment to a solid phase or to another molecule or other moiety. Such attachment can be formed by synthesizing the compound or derivative on the linker attached to a solid phase medium e.g., in a combinatorial synthesis in a plurality of compound. Likewise, the attachment to a solid phase medium can provide an affinity medium (e.g., for affinity chromatography).

[0074]   The attachment component can also include a label, which can be a directly detectable label such as a fluorophore, or an indirectly detectable such as a member of a specific binding pair, e.g., biotin.

[0075]   The ability to identify energentically allowed sites on a PDE5A binding compound, also, in a related aspect, provides modified binding compounds that have linkers attached, preferably at an energetically allowed site for binding of the modified compound to PDE5A. The linker can be attached to an attachment component as described above.

[0076]   Another aspect concerns a modified PDE5A polypeptide that includes a modification that makes the modified PDE5A more similar than native PDE5A to another phosphodiesterase, and can also include other mutations or other modifications. In various embodiments, the polypeptide includes a full-length PDE5A polypeptide, includes a modified PDE5A binding site, includes at least 20, 30, 40, 50, 60, 70, or 80 contiguous amino acid residues derived from PDE5A including a conserved site.

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[0077] Still another aspect of the invention concerns a method for developing a ligand for a phosphodiesterase that includes conserved residues matching any one, 2, 3, 4, 5, or 6 of conserved PDE5A active site residues, by determining whether a compound binds to the phosphodiesterase and interacts with such active site residues in a PDE5A crystal. The method can also include determining whether the compound modulates the activity of the phosphodiesterase. Preferably the phosphodiesterase has at least 50, 55, 60, or 70% identity over an equal length phosphodiesterase domain segment.

[0078] In particular embodiments, the determining includes computer fitting the compound in a binding site of the phosphodiesterase and/or the method includes forming a co-crystal of the phosphodiesterase and the compound. Such co-crystals can be used for determing the binding orientation of the compound with the phosphodiesterase and/or provide structural information on the phosphodiesterase, e.g., on the binding site and interacting amino acid residues. Such binding orientation and/or other structural information can be accomplished using X-ray crystallography.

[0079] The invention also provides compounds that bind to and/or modulate (*e.g.,* inhibit) PDE5A, *e.g.,* PDE5A phosphodiesterase activity. Accordingly, in aspects and embodiments involving PDE5A binding compounds, molecular scaffolds, and ligands or modulators, the compound is a weak binding compound; a moderate binding compound; a strong binding compound; the compound interacts with one or more conserved PDE5A active site residues; the compound is a small molecule; the compound binds to a plurality of different phosphodiesterases (*e.g.,* at least 2, 3, 4, 5, 7, 10, or more different phosphodiesterases).

[0080] Additional aspects and embodiments will be apparent from the following Detailed Description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0081] FIGURE 1 shows a ribbon diagram schematic representation of PDE5A phosphodiesterase domain having the sequence in Table 2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0082] The Tables will first be briefly described.

[0083] Table 1 provides atomic coordinates for human PDE5A phosphodiesterase domain. In this table, the various columns have the following content, beginning with the left-most column:

ATOM: Refers to the relevant moeity for the table row.

Atom number: Refers to the arbitrary atom number designation within the coordinate table.

Atom Name: Identifier for the atom present at the particular coordinates.

Chain ID: Chain ID refers to one monomer of the protein in the crystal, *e.g.*, chain "A", or to other compound present in the crystal, e.g., HOH for water, and L for a ligand or binding compound. Multiple copies of the protein monomers will have different chain Ids.

Residue Number: The amino acid residue number in the chain.

X, Y, Z: Respectively are the X, Y, and Z coordinate values.

Occupancy: Describes the fraction of time the atom is observed in the crystal. For example, occupancy = 1 means that the atom is present all the time; occupancy = o.5 indicates that the atom is present in the location 50% of the time.

B-factor: A measure of the thermal motion of the atom.

Element: Identifier for the element.

[0084] Table 2 provides amino acid and nucleic acid sequences for a PDE5A phosphodiesterase domain. Numbering on the amino acid sequence does not correspond to standard numbering for native PDE5A.

[0085] Table 3 provides an alignment of phosphodiesterase domains for several phosphodiesterases, including human PDE5A, providing identification of residues conserved between various members of the set.

[0086] Table 4 provides the nucleic acid and amino acid sequences for human PDE5A phosphodiesterase.

I. General and PDE5 Inhibitors

[0087] The present invention concerns the use of PDE5A phosphodiesterase structures, structural information, and related compositions for identifying compounds that modulate PDE5A phosphodiesterase activity and for determining structuctures of other phosphodiesterases.

[0088] PDE5A is involved in a number of disease and conditions, and thus can be targeted in therapeutic and prophylactic methods.

[0089] A large number of compounds that are active on PDE5, from several different chemical classes, have been identified, and pharmaceutical products directed to PDE5 have been developed and approved by the Food and Drug Administration. Such compounds can be used in conjuction with crystal structure information on PDE5A to develop improved inhibitors.

[0090] The following are among the examples of descriptions of such compounds. The compounds described in the publications listed can be used in the present invention to develop improved PDE5 inhibitors, *e.g.*, inhibitors with improved affinity, activity, and/or specificity properties. Bunnage et al., U.S. Patent 6,333,330, U.S. Patent 6,407,114, and U.S. Patent Publication 2001/0039271, all entitled PYRAZOLOPYRIMIDINONE CGMP PDE5 INHIBITORS FOR THE TREATEMENT OF SEXUAL DYSFUNCTION, describe some pyrazolopyrimidinone compounds and their synthesis, preparation of pharmaceutical compositions, and administration. Fryburg et al., U.S. Patent Application Publication 2002/0165237, entitled TREATMENT OF THE INSULIN RESISTANCE SYNDROME, lists a variety of PDE5 inhibitors, including compounds described in EP-A-0463756, EP-A-0526004, WO 93/06104, 93/07149, WO 93/12095. WO 94/00453, WO 98/49166, WO 99/54333, EP-A-0995751, WO 00/24745, EP-A-995750, WO 95/19978, and WO 93/07124, along with methods for formulating and administering pharmaceutical compositions. Bombrun, U.S. Patent 6,043,252, entitled CARBOLINE DERIVATIVES, describes PDE5 inhibitors that are carboline derivatives. Allerton, U.S. Patent Application Publication 2002/0173502, entitled PHARMACEUTICALLY ACTIVE COMPOUNDS, describes as PDE5 inhibitors certain compounds that include four heterocyclic groups. Sperl et al., U.S. Patent 6,066,634 describes substituted condensation products of N-benzyl-3-indenylacetamides herocyclic aldehydes and their use in treatment of neoplasias. Additional PDE5 inhibitors are described in Maw, U.S. Patent 6,503,908; Maw et al., U.S.

Patent 6,440,982; Daugan et al., U.S. Patent 6,143,757; Daugan et al., U.S. Patent 6,143,746; Gonzalez et al., U.S. Patent Application Publication 2002/0058606. Benzimidazole derivatives with PDE5 inhibitor activity, and their preparation and use are described in Yamasaki et al., U.S. Patent 6,166,219. All of the above references are incorporated herein by reference in their entireties.

Exemplary Diseases Associated with PDE5A.

[0091] PDE5A has been correlated with several conditions in which inhibition of PDE5A is useful. Best known is the involvement of PDE5A in treatment of erectile dysfunction. Erection is largely a haemodynamic event that is regulated by fascular tone and blood-flow balance in the penis. Because cGMP levels modulate vascular tone, PDE5A is a useful target for intervention. When a man is sexually stimulated, nitric oxide (NO) is released from non-cholinergic, non-adrenergic neurons in the penis as well as from endothelial cells. NO diffuses into cells, where it activates soluble guanylyl cyclase, the enzyme that converts GTP to cGMP. The cGMP then stimulates PKG, which initiates a protein phosphorylation cascade. This results in a descrease in intracellular levels of cancium oins, leading ultimately to dilation of the arteries that bring blood to the penis and compression o the spongy corpus-cavernosum tissue. This compression contracts veins, which reduces the outflow of blood and increases intracavernosal pressure resulting in an erection. A PDE5A inhibitor retards enzymatic hydrolysis of cGMP in the corpus cavernosum, leading to the same outcome. (Rotella, 2002, Phosphodiesterase 5 inhibitors: Current status and potential applications, *Nature Reviews* 1:674-682.) (See also, Taher et al., *J. Urol.* 149:285A (1993); Murray, *DN&P* 6(3):150-156 (1993); Emmick et al., U.S. Patent 6,451,807, entitled METHODS OF TREATING SEXUAL DYSFUNCTION IN AN INDIVIDUAL SUFFEREING FROM A RETINAL DISEASE, CLASS 1 CONGESTIVE HEART FAILURE, OR MYOCARDIAL INFARCTION USING A PDE5 INHBITOR.)

[0092] In addition to treating erectile dysfunction, PDE inhibitors are described for use in treatment of premature ejaculation in individuals with normal erectile function. Boolell, U.S. Patent Application Publication 2002/0091129.

[0093] The use of PDE5A inhibitors in treatment of cystic fibrosis has also been indicated.

[0094] Treatment of Parkinson's Disease (PD) using PDE5 inhibitors has also been indicated. For example, Roylance, U.S. Patent 6,492,371, indicates that PDE5 inhibitors are useful in methods for preventing and/or slowing the progression of PD or reducing or eliminating clinical symptoms of PD.

[0095] Watkins et al., U.S. Patent Application Publication 2002/0128171 describes the use of PDE5 inhibitors to treat gastrointestinal disorders, such as disorders characterized by hypomobility or hypermobility of small intesting, large intestine, colon, esphagus, or stomach.

[0096] The vasodilatory effects of PDE5A inhibitors allows their use in connection with some circulatory disorders. In conjunction with a prostaglandin analogue (e.g., iloprost), a PDE5A inhibitor can enhance reduction of pulmonary arterial pressure, allowing such use in patients with pulmonary hypertension.

[0097] Subarachnoid haemorrhage is a significant cause of stroke in many patients. It often occurs as a consequence of reduced responsiveness to NO in cerebral srteries. To counter this effect, PDE5A inhibitors can elevate cellular levels of cGMP in cerbral arteries, thereby at least partially correcting the vascular dysfunction.

[0098] Shahinpoor et al., U.S. Patent Application Publication 2002/0168424 describes the use of PDE5 inhibitors in conjunction with a nitric oxid donor for treatment of glaucoma. The publication indicates the drugs work synergistically to reduce intraocular pressure.

[0099] PDE5A inhibitors also moderate platelet aggregation in a dose-dependent manner.

[0100] Fryburg et al., U.S. Patent Application Publication 2002/0165,237, entitled TREATEMENT OF THE INSULIN RESISTANCE SYNDROME, describes the use of selective PDE5 inhibitors in the curative, palliative, or prophylactic treatment of insulin resistance syndrome (also referred to as Syndrome X and Metabolic Syndrome). Insulin resistance syndrome means the concomitant existence of two or more of: dyslipidemia, hypertension, type 2 diabetes mellitus or a family history of type 2 diabetes mellitus, hyperuricaemia, and/or gout, a pro-coagulant state, atheroslerosis, truncal obesity.

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[0101] Thompson et al., U.S. Patent 6,130,053, entitled METHODS FOR SELECTING COMPOUNDS FOR INHIBITON OF NEOPLASTIC LESIONS, and Thompson et al., U.S. Patent Application Publication 2002/0009764, entitled METHODS FOR IDENTIFYING COMPOUNDS FOR INHIBITON OF NEOPLASTIC LESIONS, AND PHARMACEUTICAL COMPOSITIONS CONTAINING SUCH COMPOUNDS describes the use of PDE5 inhibitors in conjunction with inhibition of PDE2 activity, leading to cell apoptosis, and methods for identifying useful compounds. See also, Pamakcu et al., U.S. Patent 6,500,610, entitled METHODS FOR IDENTIFYING COMPOUNDS FOR INHIBITING NEOPLASTIC LESIONS, AND PHARMACEUTICAL COMPOSITIONS CONTAINING SUCH COMPOUNDS. Similarly, Whitehead, U.S. Patent 6,479,493 describes the use of PDE2 inhibition combined with PDE5 inhibition for treatment of Type 1 diabetes, and describes compouns fo that purpose. Use of combination PDE2 and PDE5 inhibition is also described in Earle et al., U.S. Patent 6,465,494, entitled METHODS FOR TREATMENT OF CYSTIC FIBROSIS.

[0102] Bombrun, U.S. Patent 6,043,252 indicates that PDE5 inhibitors are useful for treatment of stable, unstable, and variant (Prinzmetal) angina, hypertension, pulmonary hypertenion, chronic obstructive pulmonary disease, congestive heart failure, acute respiratory distress syndrome, acute and chronic renal failure, atherosclerosis, conditions of reduced blood vessel patency (e.g., post-PTCA or post-bypass graft stenosis), peripheral vascular disease, vascular disorders such as Raynaud's disease, myocardial infarction, prophylaxis of stroke, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, hypertrophy, , male and female erictile dysfunction, and diseases characterized by disorders of gut motility.

[0103] Davies et al., U.S. Patent Application Publication 2002/0065286 describes the use of PDE5 inhibitors in wound treatment, such chronic wounds of non-diabetic origin, as well as acute wounds, such as in the elderly.

[0104] The present methods can be used for developing ligands for treating one or more of the diseases and conditions above, or for other diseases or conditions for which PDE5A modulation is found useful.

II.   Crystalline PDE5A

[0105] Crystalline PDE5A (*e.g.*, human PDE5A) include native crystals, phosphodiesterase domain crystals, derivative crystals and co-crystals. The native crystals generally comprise substantially pure polypeptides corresponding to PDE5A in crystalline form. PDE5A phosphodiesterase domain crystals generally comprise substantially pure PDE5A phosphodiesterase domain in crystalline form. In connection with the development of inhibitors of PDE5A phosphodiesterase function, it is advantageous to use PDE5A phosphodiesterase domain for structural determination, because use of the reduced sequence simplifies structure determination. To be useful for this purpose, the phosphodiesterase domain should be active and/or retain native-type binding, thus indicating that the phosphodiesterase domain takes on substantially normal 3D structure.

[0106] It is to be understood that the crystalline phosphodiesterases and phosphodiesterase domains of the invention are not limited to naturally occurring or native phosphodiesterase. Indeed, the crystals of the invention include crystals of mutants of native phosphodiesterases. Mutants of native phosphodiesterases are obtained by replacing at least one amino acid residue in a native phosphodiesterase with a different amino acid residue, or by adding or deleting amino acid residues within the native polypeptide or at the N- or C-terminus of the native polypeptide, and have substantially the same three-dimensional structure as the native phosphodiesterase from which the mutant is derived.

[0107] By having substantially the same three-dimensional structure is meant having a set of atomic structure coordinates that have a root-mean-square deviation of less than or equal to about 2Å when superimposed with the atomic structure coordinates of the native phosphodiesterase from which the mutant is derived when at least about 50% to 100% of the Cα atoms of the native phosphodiesterase domain are included in the superposition.

[0108] Amino acid substitutions, deletions and additions which do not significantly interfere with the three-dimensional structure of the phosphodiesterase will depend, in part, on the region of the phosphodiesterase where the substitution, addition or deletion occurs. In highly variable regions of the molecule, non-conservative substitutions as well as conservative substitutions may be tolerated without significantly disrupting the three-dimensional, structure of the molecule. In highly conserved regions, or regions containing significant secondary structure, conservative amino acid substitutions are preferred. Such

conserved and variable regions can be identified by sequence alignment of PDE5A with other phosphodiesterases. Such alignment of PDE5A phosphodiesterase domain along with a number of other phosphodiesterase domains is provided in Table 3.

[0109] Conservative amino acid substitutions are well known in the art, and include substitutions made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the amino acid residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. Other conservative amino acid substitutions are well known in the art.

[0110] For phosphodiesterases obtained in whole or in part by chemical synthesis, the selection of amino acids available for substitution or addition is not limited to the genetically encoded amino acids. Indeed, the mutants described herein may contain non-genetically encoded amino acids. Conservative amino acid substitutions for many of the commonly known non-genetically encoded amino acids are well known in the art. Conservative substitutions for other amino acids can be determined based on their physical properties as compared to the properties of the genetically encoded amino acids.

[0111] In some instances, it may be particularly advantageous or convenient to substitute, delete and/or add amino acid residues to a native phosphodiesterase in order to provide convenient cloning sites in cDNA encoding the polypeptide, to aid in purification of the polypeptide, and for crystallization of the polypeptide. Such substitutions, deletions and/or additions which do not substantially alter the three dimensional structure of the native phosphodiesterase domain will be apparent to those of ordinary skill in the art.

[0112] It should be noted that the mutants contemplated herein need not all exhibit phosphodiesterase activity. Indeed, amino acid substitutions, additions or deletions that interfere with the phosphodiesterase activity but which do not significantly alter the three-dimensional structure of the domain are specifically contemplated by the invention. Such crystalline polypeptides, or the atomic structure coordinates obtained therefrom, can be

used to identify compounds that bind to the native domain. These compounds can affect the activity of the native domain.

[0113] The derivative crystals of the invention can comprise a crystalline phosphodiesterase polypeptide in covalent association with one or more heavy metal atoms. The polypeptide may correspond to a native or a mutated phosphodiesterase. Heavy metal atoms useful for providing derivative crystals include, by way of example and not limitation, gold, mercury, selenium, etc.

[0114] The co-crystals of the invention generally comprise a crystalline phosphodiesterase domain polypeptide in association with one or more compounds. The association may be covalent or non-covalent. Such compounds include, but are not limited to, cofactors, substrates, substrate analogues, inhibitors, allosteric effectors, etc.

[0115] Exemplary mutations for PDE5A family phosphodiesterases include mutations making the phosphodiesterase active site more like the active site of PDE6 or PDE11. Such insertion is useful, for example, to assist in using PDE5A to model PDE6 or PDE11. Mutations at other sites can likewise be carried out, *e.g.*, to make a mutated PDE5A more similar to another phosphodiesterase for structure modeling and/or compound fitting purposes, such as a phosphodiesterase in the phosphodiesterase domain alignment in Table 3.

[0116] In addition to the PDE5A crystal structure described herein, a crystal-based structure of PDE5A catalytic domain is described in Brown et al., PCT Application PCT/IB02/04426, International Publication WO 03/038080. That structure (and associated atomic coordinate sets), as well as other structures and atomic coordinate sets that may be obtained can also be used as described herein.

III. Three Dimensional Structure Determination Using X-ray Crystallography

[0117] X-ray crystallography is a method of solving the three dimensional structures of molecules. The structure of a molecule is calculated from X-ray diffraction patterns using a crystal as a diffraction grating. Three dimensional structures of protein molecules arise from crystals grown from a concentrated aqueous solution of that protein. The process of X-ray crystallography can include the following steps:

    (a)   synthesizing and isolating (or otherwise obtaining) a polypeptide;

(b)   growing a crystal from an aqueous solution comprising the polypeptide with or without a modulator; and

(c)   collecting X-ray diffraction patterns from the crystals, determining unit cell dimensions and symmetry, determining electron density, fitting the amino acid sequence of the polypeptide to the electron density, and refining the structure.

Production of Polypeptides

[0118]   The native and mutated phosphodiesterase polypeptides described herein may be chemically synthesized in whole or part using techniques that are well-known in the art (*see, e.g.*, Creighton (1983) *Biopolymers* 22(1):49-58).

[0119]   Alternatively, methods which are well known to those skilled in the art can be used to construct expression vectors containing the native or mutated phosphodiesterase polypeptide coding sequence and appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis, T (1989). Molecular cloning: A laboratory Manual. Cold Spring Harbor Laboratory, New York. Cold Spring Harbor Laboratory Press; and Ausubel, F.M. et al. (1994) Current Protocols in Molecular Biology. John Wiley & Sons, Secaucus, N.J.

[0120]   A variety of host-expression vector systems may be utilized to express the phosphodiesterase coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the phosphodiesterase domain coding sequence; yeast transformed with recombinant yeast expression vectors containing the phosphodiesterase domain coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the phosphodiesterase domain coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the phosphodiesterase domain coding sequence; or animal cell systems. The expression elements of these systems vary in their strength and specificities.

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[0121] Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (*e.g.*, heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or from plant viruses (*e.g.*, the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the phosphodiesterase domain DNA, SV4O-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

[0122] Exemplary methods describing methods of DNA manipulation, vectors, various types of cells used, methods of incorporating the vectors into the cells, expression techniques, protein purification and isolation methods, and protein concentration methods are disclosed in detail in PCT publication WO 96/18738. This publication is incorporated herein by reference in its entirety, including any drawings. Those skilled in the art will appreciate that such descriptions are applicable to the present invention and can be easily adapted to it.

Crystal Growth

[0123] Crystals are grown from an aqueous solution containing the purified and concentrated polypeptide by a variety of techniques. These techniques include batch, liquid, bridge, dialysis, vapor diffusion, and hanging drop methods. McPherson (1982) John Wiley, New York; McPherson (1990) *Eur. J. Biochem.* 189:1-23; Webber (1991) *Adv. Protein Chem.* 41:1-36, incorporated by reference herein in their entireties, including all figures, tables, and drawings.

[0124] The native crystals of the invention are, in general, grown by adding precipitants to the concentrated solution of the polypeptide. The precipitants are added at a

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concentration just below that necessary to precipitate the protein. Water is removed by controlled evaporation to produce precipitating conditions, which are maintained until crystal growth ceases.

[0125]   For crystals of the invention, exemplary crystallization conditions are described in the Examples. Those of ordinary skill in the art will recognize that the exemplary crystallization conditions can be varied. Such variations may be used alone or in combination. In addition, other crystallization conditions may be found, *e.g.*, by using crystallization screening plates to identify such other conditions. Those alternate conditions can then be optimized if needed to provide larger or better quality crystals.

[0126]   Derivative crystals of the invention can be obtained by soaking native crystals in mother liquor containing salts of heavy metal atoms. It has been found that soaking a native crystal in a solution containing about 0.1 mM to about 5 mM thimerosal, 4-chloromeruribenzoic acid or $KAu(CN)_2$ for about 2 hr to about 72 hr provides derivative crystals suitable for use as isomorphous replacements in determining the X-ray crystal structure of PDE5A.

[0127]   Co-crystals of the invention can be obtained by soaking a native crystal in mother liquor containing compound that binds the phosphodiesterase, or can be obtained by co-crystallizing the phosphodiesterase polypeptide in the presence of a binding compound.

[0128]   Generally, co-crystallization of phosphodiesterase and binding compound can be accomplished using conditions identified for crystallizing the corresponding phosphodiesterase without binding compound. It is advantageous if a plurality of different crystallization conditions have been identified for the phosphodiesterase, and these can be tested to determine which condition gives the best co-crystals. It may also be benficial to optimize the conditions for co-crystallization. Alternatively, new crystallization conditions can be determined for obtaining co-crystals, *e.g.*, by screening for crystallization and then optimizing those conditions. Exemplary co-crystallization conditions are provided in the Examples.

Determining Unit Cell Dimensions and the Three Dimensional Structure of a Polypeptide or Polypeptide Complex

[0129] Once the crystal is grown, it can be placed in a glass capillary tube or other mounting device and mounted onto a holding device connected to an X-ray generator and an X-ray detection device. Collection of X-ray diffraction patterns are well documented by those in the art. See, *e.g.*, Ducruix and Geige, (1992), IRL Press, Oxford, England, and references cited therein. A beam of X-rays enters the crystal and then diffracts from the crystal. An X-ray detection device can be utilized to record the diffraction patterns emanating from the crystal. Although the X-ray detection device on older models of these instruments is a piece of film, modern instruments digitally record X-ray diffraction scattering. X-ray sources can be of various types, but advantageously, a high intensity source is used, *e.g.*, a synchrotron beam source.

[0130] Methods for obtaining the three dimensional structure of the crystalline form of a peptide molecule or molecule complex are well known in the art. See, *e.g.*, Ducruix and Geige, (1992), IRL Press, Oxford, England, and references cited therein. The following are steps in the process of determining the three dimensional structure of a molecule or complex from X-ray diffraction data.

[0131] After the X-ray diffraction patterns are collected from the crystal, the unit cell dimensions and orientation in the crystal can be determined. They can be determined from the spacing between the diffraction emissions as well as the patterns made from these emissions. The unit cell dimensions are characterized in three dimensions in units of Angstroms (one $\text{Å} = 10^{-10}$ meters) and by angles at each vertices. The symmetry of the unit cell in the crystals is also characterized at this stage. The symmetry of the unit cell in the crystal simplifies the complexity of the collected data by identifying repeating patterns. Application of the symmetry and dimensions of the unit cell is described below.

[0132] Each diffraction pattern emission is characterized as a vector and the data collected at this stage of the method determines the amplitude of each vector. The phases of the vectors can be determined using multiple techniques. In one method, heavy atoms can be soaked into a crystal, a method called isomorphous replacement, and the phases of the vectors can be determined by using these heavy atoms as reference points in the X-ray analysis. (Otwinowski, (1991), Daresbury, United Kingdom, 80-86). The isomorphous replacement method usually utilizes more than one heavy atom derivative.

[0133] In another method, the amplitudes and phases of vectors from a crystalline polypeptide with an already determined structure can be applied to the amplitudes of the vectors from a crystalline polypeptide of unknown structure and consequently determine the phases of these vectors. This second method is known as molecular replacement and the protein structure which is used as a reference must have a closely related structure to the protein of interest. (Naraza (1994) *Proteins* 11:281-296). Thus, the vector information from a phosphodiesterase of known structure, such as those reported herein, are useful for the molecular replacement analysis of another phosphodiesterase with unknown structure.

[0134] Once the phases of the vectors describing the unit cell of a crystal are determined, the vector amplitudes and phases, unit cell dimensions, and unit cell symmetry can be used as terms in a Fourier transform function. The Fourier transform function calculates the electron density in the unit cell from these measurements. The electron density that describes one of the molecules or one of the molecule complexes in the unit cell can be referred to as an electron density map. The amino acid structures of the sequence or the molecular structures of compounds complexed with the crystalline polypeptide may then be fitted to the electron density using a variety of computer programs. This step of the process is sometimes referred to as model building and can be accomplished by using computer programs such as Turbo/FRODO or "O". (Jones (1985) *Methods in Enzymology* 115:157-171).

[0135] A theoretical electron density map can then be calculated from the amino acid structures fit to the experimentally determined electron density. The theoretical and experimental electron density maps can be compared to one another and the agreement between these two maps can be described by a parameter called an R-factor. A low value for an R-factor describes a high degree of overlapping electron density between a theoretical and experimental electron density map.

[0136] The R-factor is then minimized by using computer programs that refine the theoretical electron density map. A computer program such as X-PLOR can be used for model refinement by those skilled in the art. (Brünger (1992) *Nature* 355:472-475.) Refinement may be achieved in an iterative process. A first step can entail altering the conformation of atoms defined in an electron density map. The conformations of the

atoms can be altered by simulating a rise in temperature, which will increase the vibrational frequency of the bonds and modify positions of atoms in the structure. At a particular point in the atomic perturbation process, a force field, which typically defines interactions between atoms in terms of allowed bond angles and bond lengths, Van der Waals interactions, hydrogen bonds, ionic interactions, and hydrophobic interactions, can be applied to the system of atoms. Favorable interactions may be described in terms of free energy and the atoms can be moved over many iterations until a free energy minimum is achieved. The refinement process can be iterated until the R-factor reaches a minimum value.

[0137] The three dimensional structure of the molecule or molecule complex is described by atoms that fit the theoretical electron density characterized by a minimum R-value. A file can then be created for the three dimensional structure that defines each atom by coordinates in three dimensions. An example of such a structural coordinate file is shown in Table 1.

IV. Structures of PDE5A

[0138] The present invention provides high-resolution three-dimensional structures and atomic structure coordinates of crystalline PDE5A phosphodiesterase domain and PDE5A phosphodiesterase domain co-complexed with exemplary binding compounds as determined by X-ray crystallography. The methods used to obtain the structure coordinates are provided in the examples. The atomic structure coordinates of crystalline PDE5A are listed in Table 1. Co-crystal coordinates can be used in the same way, *e.g.*, in the various aspects described herein, as coordinates for the protein by itself.

[0139] Those having skill in the art will recognize that atomic structure coordinates as determined by X-ray crystallography are not without error. Thus, it is to be understood that any set of structure coordinates obtained for crystals of PDE5A, whether native crystals, phosphodiesterase domain crystals, derivative crystals or co-crystals, that have a root mean square deviation ("r.m.s.d.") of less than or equal to about 1.5 Å when superimposed, using backbone atoms (N, C_α, C and 0), on the structure coordinates listed in Table 1 are considered to be identical with the structure coordinates listed in the Table 1 when at least about 50% to 100% of the backbone atoms of PDE5A are included in the superposition.

[0140]   As indicated above, a crystal-based PDE5A catalytic domain structure is described in Brown et al., PCT Application PCT/IB02/04426, International Publication WO 03/038080.

V.   Uses of the Crystals and Atomic Structure Coordinates

[0141]   The crystals of the invention, and particularly the atomic structure coordinates obtained therefrom, have a wide variety of uses. For example, the crystals described herein can be used as a starting point in any of the methods of use for phosphodiesterases known in the art or later developed. Such methods of use include, for example, identifying molecules that bind to the native or mutated catalytic domain of phosphodiesterases. The crystals and structure coordinates are particularly useful for identifying ligands that modulate phosphodiesterase activity as an approach towards developing new therapeutic agents. In particular, the crystals and structural information are useful in methods for ligand development utilizing molecular scaffolds.

[0142]   The structure coordinates described herein can be used as phasing models for determining the crystal structures of additional phosphodiesterases, as well as the structures of co-crystals of such phosphodiesterases with ligands such as inhibitors, agonists, antagonists, and other molecules. The structure coordinates, as well as models of the three-dimensional structures obtained therefrom, can also be used to aid the elucidation of solution-based structures of native or mutated phosphodiesterases, such as those obtained via NMR.

VI.   Electronic Representations of Phosphodiesterase Structures

[0143]   Structural information of phosphodiesterases or portions of phosphodiesterases (*e.g.*, phosphodiesterase active sites) can be represented in many different ways. Particularly useful are electronic representations, as such representations allow rapid and convenient data manipulations and structural modifications. Electronic representations can be embedded in manydifferent storage or memory media, frequently computer readable media. Examples include without limitations, computer random access memory (RAM), floppy disk, magnetic hard drive, magnetic tape (analog or digital), compact disk (CD), optical disk, CD-ROM, memory card, digital video disk (DVD), and others. The storage medium can be separate or part of a computer system. Such a computer system may be a

dedicated, special purpose, or embedded system, such as a computer system that forms part of an X-ray crystallography system, or may be a general purpose computer (which may have data connection with other equipment such as a sensor device in an X-ray crystallographic system. In many cases, the information provided by such electronic representations can also be represented physically or visually in two or three dimensions, *e.g.*, on paper, as a visual display (*e.g.*, on a computer monitor as a two dimensional or pseudo-three dimensional image) or as a three dimensional physical model. Such physical representations can also be used, alone or in connection with electronic representations. Exemplary useful representations include, but are not limited to, the following:

Atomic Coordinate Representation

[0144] One type of representation is a list or table of atomic coordinates representing positions of particular atoms in a molecular structure, portions of a structure, or complex (*e.g.*, a co-crystal). Such a representation may also include additional information, for example, information about occupancy of particular coordinates. One such atomic coordinate representation contains the coordinate information of Table 1 in electronic form.

Energy Surface or Surface of Interaction Representation

[0145] Another representation is an energy surface representation, *e.g.*, of an active site or other binding site, representing an energy surface for electronic and steric interactions. Such a representation may also include other features. An example is the inclusion of representation of a particular amino acid residue(s) or group(s) on a particular amino acid residue(s), *e.g.*, a residue or group that can participate in H-bonding or ionic interaction. Such energy surface representations can be readily generated from atomic coordinate representations using any of a variety of available computer programs.

Structural Representation

[0146] Still another representation is a structural representation, *i.e.*, a physical representation or an electronic representation of such a physical representation. Such a structural representation includes representations of relative positions of particular features of a molecule or complex, often with linkage between structural features. For example, a structure can be represented in which all atoms are linked; atoms other than hydrogen are linked; backbone atoms, with or without representation of sidechain atoms that could

participate in significant electronic interaction, are linked; among others. However, not all features need to be linked. For example, for structural representations of portions of a molecule or complex, structural features significant for that feature may be represented (*e.g.*, atoms of amino acid residues that can have significant binding interation with a ligand at a binding site. Those amino acid residues may not be linked with each other.

[0147] A structural representation can also be a schematic representation. For example, a schematic representation can represent secondary and/or tertiary structure in a schematic manner. Within such a schematic representation of a polypeptide, a particular amino acid residue(s) or group(s) on a residue(s) can be included, *e.g.*, conserved residues in a binding site, and/or residue(s) or group(s) that may interact with binding compounds. Electronic structural representations can be generated, for example, from atomic coordinate information using computer programs designed for that function and/or by constructing an electronic representation with manual input based on interpretation of another form of structural information. Physical representations can be created, for example, by printing an image of a computer-generated image or by constructing a 3D model. An example of such a printed representation is the ribbon diagram presented in Figure 1.

VII. Structure Determination for Phosphodiesterases with Unknown Structure Using Structural Coordinates

[0148] Structural coordinates, such as those set forth in Table 1, can be used to determine the three dimensional structures of phosphodiesterases with unknown structure. The methods described below can apply structural coordinates of a polypeptide with known structure to another data set, such as an amino acid sequence, X-ray crystallographic diffraction data, or nuclear magnetic resonance (NMR) data. Preferred embodiments of the invention relate to determining the three dimensional structures of other PDE5A phosphodiesterases, other phosphodiesterases, and related polypeptides.

Structures Using Amino Acid Homology

[0149] Homology modeling is a method of applying structural coordinates of a polypeptide of known structure to the amino acid sequence of a polypeptide of unknown structure. This method is accomplished using a computer representation of the three dimensional structure of a polypeptide or polypeptide complex, the computer representation of amino acid sequences of the polypeptides with known and unknown

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structures, and standard computer representations of the structures of amino acids. Homology modeling generally involves (a) aligning the amino acid sequences of the polypeptides with and without known structure; (b) transferring the coordinates of the conserved amino acids in the known structure to the corresponding amino acids of the polypeptide of unknown structure; refining the subsequent three dimensional structure; and (d) constructing structures of the rest of the polypeptide. One skilled in the art recognizes that conserved amino acids between two proteins can be determined from the sequence alignment step in step (a).

[0150]   The above method is well known to those skilled in the art. (Greer (1985) *Science* 228:1055; Blundell et *al.* A(1988) *Eur. J. Biochem.* 172:513. An exemplary computer program that can be utilized for homology modeling by those skilled in the art is the Homology module in the Insight II modeling package distributed by Accelerys Inc.

[0151]   Alignment of the amino acid sequence is accomplished by first placing the computer representation of the amino acid sequence of a polypeptide with known structure above the amino acid sequence of the polypeptide of unknown structure. Amino acids in the sequences are then compared and groups of amino acids that are homologous (e.g., amino acid side chains that are similar in chemical nature - aliphatic, aromatic, polar, or charged) are grouped together. This method will detect conserved regions of the polypeptides and account for amino acid insertions or deletions. Such alignment and/or can also be performed fully electronically using sequence alignment and analyses software.

[0152]   Once the amino acid sequences of the polypeptides with known and unknown structures are aligned, the structures of the conserved amino acids in the computer representation of the polypeptide with known structure are transferred to the corresponding amino acids of the polypeptide whose structure is unknown. For example, a tyrosine in the amino acid sequence of known structure may be replaced by a phenylalanine, the corresponding homologous amino acid in the amino acid sequence of unknown structure.

[0153]   The structures of amino acids located in non-conserved regions are to be assigned manually by either using standard peptide geometries or molecular simulation techniques, such as molecular dynamics. The final step in the process is accomplished by refining the

entire structure using molecular dynamics and/or energy minimization. The homology modeling method is well known to those skilled in the art and has been practiced using different protein molecules. For example, the three dimensional structure of the polypeptide corresponding to the catalytic domain of a serine/threonine protein kinase, myosin light chain protein kinase, was homology modeled from the cAMP-dependent protein kinase catalytic subunit. (Knighton et al. (1992) *Science* 258:130-135.)

Structures Using Molecular Replacement

[0154] Molecular replacement is a method of applying the X-ray diffraction data of a polypeptide of known structure to the X-ray diffraction data of a polypeptide of unknown sequence. This method can be utilized to define the phases describing the X-ray diffraction data of a polypeptide of unknown structure when only the amplitudes are known. X-PLOR is a commonly utilized computer software package used for molecular replacement. Brünger (1992) *Nature* 355:472-475. AMORE is another program used for molecular replacement. Navaza (1994) *Acta Crystallogr.* A50:157-163. Preferably, the resulting structure does not exhibit a root-mean-square deviation of more than 3Å.

[0155] A goal of molecular replacement is to align the positions of atoms in the unit cell by matching electron diffraction data from two crystals. A program such as X-PLOR can involve four steps. A first step can be to determine the number of molecules in the unit cell and define the angles between them. A second step can involve rotating the diffraction data to define the orientation of the molecules in the unit cell. A third step can be to translate the electron density in three dimensions to correctly position the molecules in the unit cell. Once the amplitudes and phases of the X-ray diffraction data is determined, an R-factor can be calculated by comparing electron diffraction maps calculated experimentally from the reference data set and calculated from the new data set. An R-factor between 30-50% indicates that the orientations of the atoms in the unit cell are reasonably determined by this method. A fourth step in the process can be to decrease the R-factor to roughly 20% by refining the new electron density map using iterative refinement techniques described herein and known to those or ordinary skill in the art.

Structures Using NMR Data

[0156] Structural coordinates of a polypeptide or polypeptide complex derived from X-ray crystallographic techniques can be applied towards the elucidation of three

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dimensional structures of polypeptides from nuclear magnetic resonance (NMR) data. This method is used by those skilled in the art. (Wuthrich, (1986), John Wiley and Sons, New York:176-199; Pflugrath et *al.* (1986) *J. Mol. Biol.* 189:383-386; Kline *et al.* (1986) *J. Mol. Biol.* 189:377-382.) While the secondary structure of a polypeptide is often readily determined by utilizing two-dimensional NMR data, the spatial connections between individual pieces of secondary structure are not as readily determinable. The coordinates defining a three-dimensional structure of a polypeptide derived from X-ray crystallographic techniques can guide the NMR spectroscopist to an understanding of these spatial interactions between secondary structural elements in a polypeptide of related structure.

[0157] The knowledge of spatial interactions between secondary structural elements can greatly simplify Nuclear Overhauser Effect (NOE) data from two-dimensional NMR experiments. Additionally, applying the crystallographic coordinates after the determination of secondary structure by NMR techniques only simplifies the assignment of NOEs relating to particular amino acids in the polypeptide sequence and does not greatly bias the NMR analysis of polypeptide structure. Conversely, using the crystallographic coordinates to simplify NOE data while determining secondary structure of the polypeptide would bias the NMR analysis of protein structure.

VIII. Structure-Based Design of Modulators of Phosphodiesterase Function Utilizing Structural Coordinates

[0158] Structure-based modulator design and identification methods are powerful techniques that can involve searches of computer databases containing a wide variety of potential modulators and chemical functional groups. The computerized design and identification of modulators is useful as the computer databases contain more compounds than the chemical libraries, often by an order of magnitude. For reviews of structure-based drug design and identification (*see* Kuntz et *al.* (1994), *Acc. Chem. Res.* 27:117; Guida (1994) *Current Opinion in Struc. Biol.* 4: 777; Colman (1994) *Current Opinion in Struc. Biol.* 4: 868).

[0159] The three dimensional structure of a polypeptide defined by structural coordinates can be utilized by these design methods, for example, the structural coordinates of Table 1. In addition, the three dimensional structures of phosphodiesterases

determined by the homology, molecular replacement, and NMR techniques described herein can also be applied to modulator design and identification methods.

[0160] For identifying modulators, structural information for a native phosphodiesterase, in particular, structural information for the active site of the phosphodiesterase, can be used. However, it may be advantageous to utilize structural information from one or more co-crystals of the phosphodiesterase with one or more binding compounds. It can also be advantageous if the binding compound has a structural core in common with test compounds.

Design by Searching Molecular Data Bases

[0161] One method of rational design searches for modulators by docking the computer representations of compounds from a database of molecules. Publicly available databases include, for example:

a) ACD from Molecular Designs Limited

b) NCI from National Cancer Institute

c) CCDC from Cambridge Crystallographic Data Center

d) CAST from Chemical Abstract Service

e) Derwent from Derwent Information Limited

f) Maybridge from Maybridge Chemical Company LTD

g) Aldrich from Aldrich Chemical Company

h) Directory of Natural Products from Chapman & Hall

[0162] One such data base (ACD distributed by Molecular Designs Limited Information Systems) contains compounds that are synthetically derived or are natural products. Methods available to those skilled in the art can convert a data set represented in two dimensions to one represented in three dimensions. These methods are enabled by such computer programs as CONCORD from Tripos Associates or DE-Converter from Molecular Simulations Limited.

[0163] Multiple methods of structure-based modulator design are known to those in the art. (Kuntz et al., (1982), *J. Mol. Biol. 162:* 269; Kuntz et aZ., (1994), Acc. *Chern. Res. 27:* 117; Meng et al., (1992), *J. Compt. Chem. 13:* 505; Bohm, (1994), *J. Comp. Aided Molec. Design 8:* 623.)

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[0164]   A computer program widely utilized by those skilled in the art of rational modulator design is DOCK from the University of California in San Francisco. The general methods utilized by this computer program and programs like it are described in three applications below. More detailed information regarding some of these techniques can be found in the Accelerys User Guide, 1995. A typical computer program used for this purpose can perform a processes comprising the following steps or functions:

>   (a) remove the existing compound from the protein;

>   (b) dock the structure of another compound into the active-site using the computer program (such as DOCK) or by interactively moving the compound into the active-site;

>   (c) characterize the space between the compound and the active-site atoms;

>   (d) search libraries for molecular fragments which (i) can fit into the empty space between the compound and the active-site, and (ii) can be linked to the compound; and

>   (e) link the fragments found above to the compound and evaluate the new modified compound.

[0165]   Part (c) refers to characterizing the geometry and the complementary interactions formed between the atoms of the active site and the compounds. A favorable geometric fit is attained when a significant surface area is shared between the compound and active-site atoms without forming unfavorable steric interactions.One skilled in the art would note that the method can be performed by skipping parts (d) and (e) and screening a database of many compounds.

[0166]   Structure-based design and identification of modulators of phosphodiesterase function can be used in conjunction with assay screening. As large computer databases of compounds (around 10,000 compounds) can be searched in a matter of hours or even less, the computer-based method can narrow the compounds tested as potential modulators of phosphodiesterase function in biochemical or cellular assays.

[0167]   The above descriptions of structure-based modulator design are not all encompassing and other methods are reported in the literature and can be used, *e.g.*:

>   (1) CAVEAT: Bartlett *et* al.,(1989), in Chemical and Biological Problems in Molecular Recognition, Roberts, S.M.; Ley, S.V.; Campbell, M.M. eds.; *Royal*

Society of Chemistry: Cambridge, pp.182-196.

(2) FLOG: Miller et al., (1994), *J. Comp. Aided Molec. Design* 8:153.

(3) PRO Modulator: Clark *et al.,* (1995), *J. Comp. Aided Molec. Design* 9:13.

(4) MCSS: Miranker and Karplus, (1991), *Proteins: Structure, Function, and Genetics 11:29.*

(5) AUTODOCK: Goodsell and Olson, (1990), *Proteins: Structure, Function, and Genetics 8:195.*

(6) GRID: Goodford, (1985), *J. Med. Chem.* 28:849.

Design by Modifying Compounds in Complex with PDE5A

[0168] Another way of identifying compounds as potential modulators is to modify an existing modulator in the polypeptide active site. For example, the computer representation of modulators can be modified within the computer representation of a PDE5A active site. Detailed instructions for this technique can be found, for example, in the Accelerys User Manual, 1995 in LUDI. The computer representation of the modulator is typically modified by the deletion of a chemical group or groups or by the addition of a chemical group or groups.

[0169] Upon each modification to the compound, the atoms of the modified compound and active site can be shifted in conformation and the distance between the modulator and the active-site atoms may be scored along with any complementary interactions formed between the two molecules. Scoring can be complete when a favorable geometric fit and favorable complementary interactions are attained. Compounds that have favorable scores are potential modulators.

Design by Modifying the Structure of Compounds that Bind PDE5A

[0170] A third method of structure-based modulator design is to screen compounds designed by a modulator building or modulator searching computer program. Examples of these types of programs can be found in the Molecular Simulations Package, Catalyst. Descriptions for using this program are documented in the Molecular Simulations User Guide (1995). Other computer programs used in this application are ISIS/HOST, ISIS/BASE, ISIS/DRAW) from Molecular Designs Limited and UNITY from Tripos Associates.

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[0171] These programs can be operated on the structure of a compound that has been removed from the active site of the three dimensional structure of a compound-phosphodiesterase complex. Operating the program on such a compound is preferable since it is in a biologically active conformation.

[0172] A modulator construction computer program is a computer program that may be used to replace computer representations of chemical groups in a compound complexed with a phosphodiesterase or other biomolecule with groups from a computer database. A modulator searching computer program is a computer program that may be used to search computer representations of compounds from a computer data base that have similar three dimensional structures and similar chemical groups as compound bound to a particular biomolecule.

[0173] A typical program can operate by using the following general steps:

    (a) map the compounds by chemical features such as by hydrogen bond donors or acceptors, hydrophobic/lipophilic sites, positively ionizable sites, or negatively ionizable sites;

    (b) add geometric constraints to the mapped features; and

    (c) search databases with the model generated in (b).

[0174] Those skilled in the art also recognize that not all of the possible chemical features of the compound need be present in the model of (b). One can use any subset of the model to generate different models for data base searches.

Modulator Design Using Molecular Scaffolds

[0175] The present invention can also advantageously utilize methods for designing compounds, designated as molecular scaffolds, that can act broadly across families of molecules and/or for using a molecular scaffold to design ligands that target individual or multiple members of those families. Such design using molecular scaffolds is described in Hirth and Milburn, U.S. Patent Application 10/377,268, which is incorporated herein by reference in its entirety. Such design and development using molecular scaffolds is described, in part, below.

[0176] In preferred embodiments, the molecules can be proteins and a set of chemical compounds can be assembled that have properties such that they are 1) chemically

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designed to act on certain protein families and/or 2) behave more like molecular scaffolds, meaning that they have chemical substructures that make them specific for binding to one or more proteins in a family of interest. Alternatively, molecular scaffolds can be designed that are preferentially active on an individual target molecule.

[0177] Useful chemical properties of molecular scaffolds can include one or more of the following characteristics, but are not limited thereto: an average molecular weight below about 350 daltons, or between from about 150 to about 350 daltons, or from about 150 to about 300 daltons; having a clogP below 3; a number of rotatable bonds of less than 4; a number of hydrogen bond donors and acceptors below 5 or below 4; a polar surface area of less than 50 $Å^2$; binding at protein binding sites in an orientation so that chemical substituents from a combinatorial library that are attached to the scaffold can be projected into pockets in the protein binding site; and possessing chemically tractable structures at its substituent attachment points that can be modified, thereby enabling rapid library construction.

[0178] By "clog P" is meant the calculated log P of a compound, "P" referring to the partition coefficient between octanol and water.

[0179] The term "Molecular Polar Surface Area (PSA)" refers to the sum of surface contributions of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. The polar surface area has been shown to correlate well with drug transport properties, such as intestinal absorption, or blood-brain barrier penetration.

[0180] Additional useful chemical properties of distinct compounds for inclusion in a combinatorial library include the ability to attach chemical moieties to the compound that will not interfere with binding of the compound to at least one protein of interest, and that will impart desirable properties to the library members, for example, causing the library members to be actively transported to cells and/or organs of interest, or the ability to attach to a device such as a chromatography column (e.g., a streptavidin column through a molecule such as biotin) for uses such as tissue and proteomics profiling purposes.

[0181] A person of ordinary skill in the art will realize other properties that can be desirable for the scaffold or library members to have depending on the particular requirements of the use, and that compounds with these properties can also be sought and

identified in like manner. Methods of selecting compounds for assay are known to those of ordinary skill in the art, for example, methods and compounds described in U.S. Patent No. 6,288,234, 6,090,912, 5,840,485, each of which is hereby incorporated by reference in its entirety, including all charts and drawings.

[0182] In various embodiments, the present invention provides methods of designing ligands that bind to a plurality of members of a molecular family, where the ligands contain a common molecular scaffold. Thus, a compound set can be assayed for binding to a plurality of members of a molecular family, e.g., a protein family. One or more compounds that bind to a plurality of family members can be identified as molecular scaffolds. When the orientation of the scaffold at the binding site of the target molecules has been determined and chemically tractable structures have been identified, a set of ligands can be synthesized starting with one or a few molecular scaffolds to arrive at a plurality of ligands, wherein each ligand binds to a separate target molecule of the molecular family with altered or changed binding affinity or binding specificity relative to the scaffold. Thus, a plurality of drug lead molecules can be designed to preferentially target individual members of a molecular family based on the same molecular scaffold, and act on them in a specific manner.

IX. Binding Assays

[0183] The methods of the present invention can involve assays that are able to detect the binding of compounds to a target molecule. Such binding is at a statistically significant level, preferably with a confidence level of at least 90%, more preferably at least 95, 97, 98, 99% or greater confidence level that the assay signal represents binding to the target molecule, i.e., is distinguished from background. Preferably controls are used to distinguish target binding from non-specific binding. The assays of the present invention can also include assaying compounds for low affinity binding to the target molecule. A large variety of assays indicative of binding are known for different target types and can be used for this invention. Compounds that act broadly across protein families are not likely to have a high affinity against individual targets, due to the broad nature of their binding. Thus, assays described herein allow for the identification of compounds that bind with low affinity, very low affinity, and extremely low affinity. Therefore, potency (or binding affinity) is not the primary, nor even the most important, indicia of identification of a potentially useful binding compound. Rather, even those compounds that bind with

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low affinity, very low affinity, or extremely low affinity can be considered as molecular scaffolds that can continue to the next phase of the ligand design process.

[0184] By binding with "low affinity" is meant binding to the target molecule with a dissociation constant (k_d) of greater than 1 μM under standard conditions. By binding with "very low affinity" is meant binding with a k_d of above about 100 μM under standard conditions. By binding with "extremely low affinity" is meant binding at a k_d of above about 1 mM under standard conditions. By "moderate affinity" is meant binding with a k_d of from about 200 nM to about 1 μM under standard conditions. By "moderately high affinity" is meant binding at a k_d of from about 1 nM to about 200 nM. By binding at "high affinity" is meant binding at a k_d of below about 1 nM under standard conditions. For example, low affinity binding can occur because of a poorer fit into the binding site of the target molecule or because of a smaller number of non-covalent bonds, or weaker covalent bonds present to cause binding of the scaffold or ligand to the binding site of the target molecule relative to instances where higher affinity binding occurs. The standard conditions for binding are at pH 7.2 at 37°C for one hour. For example, 100 μl/well can be used in HEPES 50 mM buffer at pH 7.2, NaCl 15 mM, ATP 2 μM, and bovine serum albumin 1 ug/well, 37°C for one hour.

[0185] Binding compounds can also be characterized by their effect on the activity of the target molecule. Thus, a "low activity" compound has an inhibitory concentration (IC_{50}) or excitation concentration (EC_{50}) of greater than 1 μM under standard conditions. By "very low activity" is meant an IC_{50} or EC_{50} of above 100 μM under standard conditions. By "extremely low activity" is meant an IC_{50} or EC_{50} of above 1 mM under standard conditions. By "moderate activity" is meant an IC_{50} or EC_{50} of 200 nM to 1 μM under standard conditions. By "moderately high activity" is meant an IC_{50} or EC_{50} of 1 nM to 200 nM. By "high activity" is meant an IC_{50} or EC_{50} of below 1 nM under standard conditions. The IC_{50} (or EC_{50}) is defined as the concentration of compound at which 50% of the activity of the target molecule (e.g., enzyme or other protein) activity being measured is lost (or gained) relative to activity when no compound is present. Activity can be measured using methods known to those of ordinary skill in the art, e.g., by measuring any detectable product or signal produced by occurrence of an enzymatic reaction, or other activity by a protein being measured.

[0186] By "background signal" in reference to a binding assay is meant the signal that is recorded under standard conditions for the particular assay in the absence of a test compound, molecular scaffold, or ligand that binds to the target molecule. Persons of ordinary skill in the art will realize that accepted methods exist and are widely available for determining background signal.

[0187] By "standard deviation" is meant the square root of the variance. The variance is a measure of how spread out a distribution is. It is computed as the average squared deviation of each number from its mean. For example, for the numbers 1, 2, and 3, the mean is 2 and the variance is:

$$\sigma^2 = \frac{(1\text{-}2)^2 + (2\text{-}2)^2 + (3\text{-}2)^2}{3} = 0.667$$

[0188] To design or discover scaffolds that act broadly across protein families, proteins of interest can be assayed against a compound collection or set. The assays can preferably be enzymatic or binding assays. In some embodiments it may be desirable to enhance the solubility of the compounds being screened and then analyze all compounds that show activity in the assay, including those that bind with low affinity or produce a signal with greater than about three times the standard deviation of the background signal. The assays can be any suitable assay such as, for example, binding assays that measure the binding affinity between two binding partners. Various types of screening assays that can be useful in the practice of the present invention are known in the art, such as those described in U.S. Patent Nos. 5,763,198, 5,747,276, 5,877,007, 6,243,980, 6,294,330, and 6,294,330, each of which is hereby incorporated by reference in its entirety, including all charts and drawings.

[0189] In various embodiments of the assays at least one compound, at least about 5%, at least about 10%, at least about 15%, at least about 20%, or at least about 25% of the compounds can bind with low affinity. In general, up to about 20% of the compounds can show activity in the screening assay and these compounds can then be analyzed directly with high-throughput co-crystallography, computational analysis to group the compounds into classes with common structural properties (e.g., structural core and/or shape and polarity characteristics), and the identification of common chemical structures between compounds that show activity.

[0190] The person of ordinary skill in the art will realize that decisions can be based on criteria that are appropriate for the needs of the particular situation, and that the decisions can be made by computer software programs. Classes can be created containing almost any number of scaffolds, and the criteria selected can be based on increasingly exacting criteria until an arbitrary number of scaffolds is arrived at for each class that is deemed to be advantageous.

Surface Plasmon Resonance

[0191] Binding parameters can be measured using surface plasmon resonance, for example, with a BIAcore® chip (Biacore, Japan) coated with immobilized binding components. Surface plasmon resonance is used to characterize the microscopic association and dissociation constants of reaction between an sFv or other ligand directed against target molecules. Such methods are generally described in the following references which are incorporated herein by reference. Vely F. et al., (2000) BIAcore® analysis to test phosphopeptide-SH2 domain interactions, *Methods in Molecular Biology.* 121:313-21; Liparoto et al., (1999) Biosensor analysis of the interleukin-2 receptor complex, *Journal of Molecular Recognition.* 12:316-21; Lipschultz et al., (2000) Experimental design for analysis of complex kinetics using surface plasmon resonance, *Methods.* 20(3):310-8; Malmqvist., (1999) BIACORE: an affinity biosensor system for characterization of biomolecular interactions, Biochemical Society Transactions 27:335-40; Alfthan, (1998) Surface plasmon resonance biosensors as a tool in antibody engineering, *Biosensors & Bioelectronics.* 13:653-63; Fivash et al., (1998) BIAcore for macromolecular interaction, Current Opinion in Biotechnology. 9:97-101; Price et al.; (1998) Summary report on the ISOBM TD-4 Workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. *Tumour Biology* 19 Suppl 1:1-20; Malmqvist et al, (1997) Biomolecular interaction analysis: affinity biosensor technologies for functional analysis of proteins, *Current Opinion in Chemical Biology.* 1:378-83; O'Shannessy et al., (1996) Interpretation of deviations from pseudo-first-order kinetic behavior in the characterization of ligand binding by biosensor technology, *Analytical Biochemistry.* 236:275-83; Malmborg et al., (1995) BIAcore as a tool in antibody engineering, *Journal of Immunological Methods.* 183:7-13; Van Regenmortel, (1994) Use of biosensors to characterize recombinant proteins, *Developments in Biological Standardization.* 83:143-51; and O'Shannessy, (1994) Determination of kinetic rate and equilibrium binding

constants for macromolecular interactions: a critique of the surface plasmon resonance literature, *Current Opinions in Biotechnology.* 5:65-71.

[0192] BIAcore® uses the optical properties of surface plasmon resonance (SPR) to detect alterations in protein concentration bound to a dextran matrix lying on the surface of a gold/glass sensor chip interface, a dextran biosensor matrix. In brief, proteins are covalently bound to the dextran matrix at a known concentration and a ligand for the protein is injected through the dextran matrix. Near infrared light, directed onto the opposite side of the sensor chip surface is reflected and also induces an evanescent wave in the gold film, which in turn, causes an intensity dip in the reflected light at a particular angle known as the resonance angle. If the refractive index of the sensor chip surface is altered (e.g., by ligand binding to the bound protein) a shift occurs in the resonance angle. This angle shift can be measured and is expressed as resonance units (RUs) such that 1000 RUs is equivalent to a change in surface protein concentration of 1 ng/mm^2. These changes are displayed with respect to time along the y-axis of a sensorgram, which depicts the association and dissociation of any biological reaction.

High Throughput Screening (HTS) Assays

[0193] HTS typically uses automated assays to search through large numbers of compounds for a desired activity. Typically HTS assays are used to find new drugs by screening for chemicals that act on a particular enzyme or molecule. For example, if a chemical inactivates an enzyme it might prove to be effective in preventing a process in a cell which causes a disease. High throughput methods enable researchers to assay thousands of different chemicals against each target molecule very quickly using robotic handling systems and automated analysis of results.

[0194] As used herein, "high throughput screening" or "HTS" refers to the rapid in vitro screening of large numbers of compounds (libraries); generally tens to hundreds of thousands of compounds, using robotic screening assays. Ultra high-throughput Screening (uHTS) generally refers to the high-throughput screening accelerated to greater than 100,000 tests per day.

[0195] To achieve high-throughput screening, it is advantageous to house samples on a multicontainer carrier or platform. A multicontainer carrier facilitates measuring reactions of a plurality of candidate compounds simultaneously. Multi-well microplates may be

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used as the carrier. Such multi-well microplates, and methods for their use in numerous assays, are both known in the art and commercially available.

[0196] Screening assays may include controls for purposes of calibration and confirmation of proper manipulation of the components of the assay. Blank wells that contain all of the reactants but no member of the chemical library are usually included. As another example, a known inhibitor (or activator) of an enzyme for which modulators are sought, can be incubated with one sample of the assay, and the resulting decrease (or increase) in the enzyme activity used as a comparator or control. It will be appreciated that modulators can also be combined with the enzyme activators or inhibitors to find modulators which inhibit the enzyme activation or repression that is otherwise caused by the presence of the known the enzyme modulator. Similarly, when ligands to a sphingolipid target are sought, known ligands of the target can be present in control/calibration assay wells.

Measuring Enzymatic and Binding Reactions During Screening Assays

[0197] Techniques for measuring the progression of enzymatic and binding reactions, e.g., in multicontainer carriers, are known in the art and include, but are not limited to, the following.

[0198] Spectrophotometric and spectrofluorometric assays are well known in the art. Examples of such assays include the use of colorimetric assays for the detection of peroxides, as disclosed in Example 1(b) and Gordon, A. J. and Ford, R. A., (1972) The Chemist's Companion: A Handbook Of Practical Data, Techniques, And References, John Wiley and Sons, N.Y., Page 437.

[0199] Fluorescence spectrometry may be used to monitor the generation of reaction products. Fluorescence methodology is generally more sensitive than the absorption methodology. The use of fluorescent probes is well known to those skilled in the art. For reviews, see Bashford et al., (1987) Spectrophotometry and Spectrofluorometry: A Practical Approach, pp. 91-114, IRL Press Ltd.; and Bell, (1981) Spectroscopy In Biochemistry, Vol. I, pp. 155-194, CRC Press.

[0200] In spectrofluorometric methods, enzymes are exposed to substrates that change their intrinsic fluorescence when processed by the target enzyme. Typically, the substrate

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is nonfluorescent and is converted to a fluorophore through one or more reactions. As a non-limiting example, SMase activity can be detected using the Amplex® Red reagent (Molecular Probes, Eugene, OR). In order to measure sphingomyelinase activity using Amplex® Red, the following reactions occur. First, SMase hydrolyzes sphingomyelin to yield ceramide and phosphorylcholine. Second, alkaline phosphatase hydrolyzes phosphorylcholine to yield choline. Third, choline is oxidized by choline oxidase to betaine. Finally, H_2O_2, in the presence of horseradish peroxidase, reacts with Amplex® Red to produce the fluorescent product, Resorufin, and the signal therefrom is detected using spectrofluorometry.

[0201] Fluorescence polarization (FP) is based on a decrease in the speed of molecular rotation of a fluorophore that occurs upon binding to a larger molecule, such as a receptor protein, allowing for polarized fluorescent emission by the bound ligand. FP is empirically determined by measuring the vertical and horizontal components of fluorophore emission following excitation with plane polarized light. Polarized emission is increased when the molecular rotation of a fluorophore is reduced. A fluorophore produces a larger polarized signal when it is bound to a larger molecule (i.e. a receptor), slowing molecular rotation of the fluorophore. The magnitude of the polarized signal relates quantitatively to the extent of fluorescent ligand binding. Accordingly, polarization of the "bound" signal depends on maintenance of high affinity binding.

[0202] FP is a homogeneous technology and reactions are very rapid, taking seconds to minutes to reach equilibrium. The reagents are stable, and large batches may be prepared, resulting in high reproducibility. Because of these properties, FP has proven to be highly automatable, often performed with a single incubation with a single, premixed, tracer-receptor reagent. For a review, see Owickiet al., (1997), Application of Fluorescence Polarization Assays in High-Throughput Screening, *Genetic Engineering News*, 17:27.

[0203] FP is particularly desirable since its readout is independent of the emission intensity (Checovich, W. J., et al., (1995) *Nature* 375:254-256; Dandliker, W. B., et al., (1981) *Methods in Enzymology* 74:3-28) and is thus insensitive to the presence of colored compounds that quench fluorescence emission. FP and FRET (see below) are well-suited for identifying compounds that block interactions between sphingolipid receptors and their ligands. See, for example, Parker et al., (2000) Development of high throughput screening

assays using fluorescence polarization: nuclear receptor-ligand-binding and kinase/phosphatase assays, J Biomol Screen 5:77-88.

[0204] Fluorophores derived from sphingolipids that may be used in FP assays are commercially available. For example, Molecular Probes (Eugene, OR) currently sells sphingomyelin and one ceramide flurophores. These are, respectively, N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene- 3-pentanoyl)sphingosyl phosphocholine (BODIPY® FL C5-sphingomyelin); N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene- 3-dodecanoyl)sphingosyl phosphocholine (BODIPY® FL C12-sphingomyelin); and N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene- 3-pentanoyl)sphingosine (BODIPY® FL C5-ceramide). U.S. Patent No. 4,150,949, (Immunoassay for gentamicin), discloses fluorescein-labelled gentamicins, including fluoresceinthiocarbanyl gentamicin. Additional fluorophores may be prepared using methods well known to the skilled artisan.

[0205] Exemplary normal-and-polarized fluorescence readers include the POLARION® fluorescence polarization system (Tecan AG, Hombrechtikon, Switzerland). General multiwell plate readers for other assays are available, such as the VERSAMAX® reader and the SPECTRAMAX® multiwell plate spectrophotometer (both from Molecular Devices).

[0206] Fluorescence resonance energy transfer (FRET) is another useful assay for detecting interaction and has been described. See, e.g., Heim et al., (1996) *Curr. Biol.* 6:178-182; Mitra et al., (1996) *Gene* 173:13-17; and Selvin et al., (1995) *Meth. Enzymol.* 246:300-345. FRET detects the transfer of energy between two fluorescent substances in close proximity, having known excitation and emission wavelengths. As an example, a protein can be expressed as a fusion protein with green fluorescent protein (GFP). When two fluorescent proteins are in proximity, such as when a protein specifically interacts with a target molecule, the resonance energy can be transferred from one excited molecule to the other. As a result, the emission spectrum of the sample shifts, which can be measured by a fluorometer, such as a fMAX multiwell fluorometer (Molecular Devices, Sunnyvale Calif.).

[0207] Scintillation proximity assay (SPA) is a particularly useful assay for detecting an interaction with the target molecule. SPA is widely used in the pharmaceutical industry and has been described (Hanselman et al., (1997) *J. Lipid Res.* 38:2365-2373; Kahl et al.,

(1996) *Anal. Biochem.* 243:282-283; Undenfriend et al., (1987) *Anal. Biochem.* 161:494-500). See also U.S. Patent Nos. 4,626,513 and 4,568,649, and European Patent No. 0,154,734. One commercially available system uses FLASHPLATE® scintillant-coated plates (NEN Life Science Products, Boston, MA).

[0208] The target molecule can be bound to the scintillator plates by a variety of well known means. Scintillant plates are available that are derivatized to bind to fusion proteins such as GST, His6 or Flag fusion proteins. Where the target molecule is a protein complex or a multimer, one protein or subunit can be attached to the plate first, then the other components of the complex added later under binding conditions, resulting in a bound complex.

[0209] In a typical SPA assay, the gene products in the expression pool will have been radiolabeled and added to the wells, and allowed to interact with the solid phase, which is the immobilized target molecule and scintillant coating in the wells. The assay can be measured immediately or allowed to reach equilibrium. Either way, when a radiolabel becomes sufficiently close to the scintillant coating, it produces a signal detectable by a device such as a TOPCOUNT NXT® microplate scintillation counter (Packard BioScience Co., Meriden Conn.). If a radiolabeled expression product binds to the target molecule, the radiolabel remains in proximity to the scintillant long enough to produce a detectable signal.

[0210] In contrast, the labeled proteins that do not bind to the target molecule, or bind only briefly, will not remain near the scintillant long enough to produce a signal above background. Any time spent near the scintillant caused by random Brownian motion will also not result in a significant amount of signal. Likewise, residual unincorporated radiolabel used during the expression step may be present, but will not generate significant signal because it will be in solution rather than interacting with the target molecule. These non-binding interactions will therefore cause a certain level of background signal that can be mathematically removed. If too many signals are obtained, salt or other modifiers can be added directly to the assay plates until the desired specificity is obtained (Nichols et al., (1998) *Anal. Biochem.* 257:112-119).

Assay Compounds and Molecular Scaffolds

[0211]   Preferred characteristics of a scaffold include being of low molecular weight (e.g., less than 350 Da, or from about 100 to about 350 daltons, or from about 150 to about 300 daltons). Preferably clog P of a scaffold is from −1 to 8, more preferably less than 6, 5, or 4, most preferably less than 3. In particular embodiments the clogP is in a range −1 to an upper limit of 2, 3, 4, 5, 6, or 8; or is in a range of 0 to an upper limit of 2,3, 4, 5, 6, or 8. Preferably the number of rotatable bonds is less than 5, more preferably less than 4. Preferably the number of hydrogen bond donors and acceptors is below 6, more preferably below 5. An additional criterion that can be useful is a polar surface area of less than 5. Guidance that can be useful in identifying criteria for a particular application can be found in Lipinski et al., (1997) *Advanced Drug Delivery Reviews* 23 3-25, which is hereby incorporated by reference in its entirety.

[0212]   ·A scaffold may preferably bind to a given protein binding site in a configuration that causes substituent moieties of the scaffold to be situated in pockets of the protein binding site. Also, possessing chemically tractable groups that can be chemically modified, particularly through synthetic reactions, to easily create a combinatorial library can be a preferred characteristic of the scaffold. Also preferred can be having positions on the scaffold to which other moieties can be attached, which do not interfere with binding of the scaffold to the protein(s) of interest but do cause the scaffold to achieve a desirable property, for example, active transport of the scaffold to cells and/or organs, enabling the scaffold to be attached to a chromatographic column to facilitate analysis, or another desirable property. A molecular scaffold can bind to a target molecule with any affinity, such as binding at high affinity, moderate affinity, low affinity, very low affinity, or extremely low affinity.

[0213]   Thus, the above criteria can be utilized to select many compounds for testing that have the desired attributes. Many compounds having the criteria described are available in the commercial market, and may be selected for assaying depending on the specific needs to which the methods are to be applied.

[0214]   A "compound library" or "library" is a collection of different compounds having different chemical structures. A compound library is screenable, that is, the compound library members therein may be subject to screening assays. In preferred embodiments,

the library members can have a molecular weight of from about 100 to about 350 daltons, or from about 150 to about 350 daltons. Examples of libraries are provided aove.

[0215]   Libraries of the present invention can contain at least one compound than binds to the target molecule at low affinity. Libraries of candidate compounds can be assayed by many different assays, such as those described above, e.g., a fluorescence polarization assay. Libraries may consist of chemically synthesized peptides, peptidomimetics, or arrays of combinatorial chemicals that are large or small, focused or nonfocused. By "focused" it is meant that the collection of compounds is prepared using the structure of previously characterized compounds and/or pharmacophores.

[0216]   Compound libraries may contain molecules isolated from natural sources, artificially synthesized molecules, or molecules synthesized, isolated, or otherwise prepared in such a manner so as to have one or more moieties variable, e.g., moieties that are independently isolated or randomly synthesized. Types of molecules in compound libraries include but are not limited to organic compounds, polypeptides and nucleic acids as those terms are used herein, and derivatives, conjugates and mixtures thereof.

[0217]   Compound libraries of the invention may be purchased on the commercial market or prepared or obtained by any means including, but not limited to, combinatorial chemistry techniques, fermentation methods, plant and cellular extraction procedures and the like (see, e.g., Cwirla et al., (1990) *Biochemistry*, 87, 6378-6382; Houghten et al., (1991) *Nature*, 354, 84-86; Lam et al., (1991) *Nature*, 354, 82-84; Brenner et al., (1992) *Proc. Natl. Acad. Sci. USA*, 89, 5381-5383; R. A. Houghten, (1993) *Trends Genet.*, 9, 235-239; E. R. Felder, (1994) *Chimia*, 48, 512-541; Gallop et al., (1994) *J. Med. Chem.*, 37, 1233-1251; Gordon et al., (1994) *J. Med. Chem.*, 37, 1385-1401; Carell et al., (1995) *Chem. Biol.*, 3, 171-183; Madden et al., *Perspectives in Drug Discovery and Design* 2, 269-282; Lebl et al., (1995) *Biopolymers*, 37 177-198); small molecules assembled around a shared molecular structure; collections of chemicals that have been assembled by various commercial and noncommercial groups, natural products; extracts of marine organisms, fungi, bacteria, and plants.

[0218]   Preferred libraries can be prepared in a homogenous reaction mixture, and separation of unreacted reagents from members of the library is not required prior to screening. Although many combinatorial chemistry approaches are based on solid state

chemistry, liquid phase combinatorial chemistry is capable of generating libraries (Sun CM., (1999) Recent advances in liquid-phase combinatorial chemistry, *Combinatorial Chemistry & High Throughput Screening*. 2:299-318).

[0219] Libraries of a variety of types of molecules are prepared in order to obtain members therefrom having one or more preselected attributes that can be prepared by a variety of techniques, including but not limited to parallel array synthesis (Houghton, (2000) *Annu Rev Pharmacol Toxicol* 40:273-82, Parallel array and mixture-based synthetic combinatorial chemistry; solution-phase combinatorial chemistry (Merritt, (1998) *Comb Chem High Throughput Screen* 1(2):57-72, Solution phase combinatorial chemistry, Coe et al., (1998-99) *Mol Divers*;4(1):31-8, Solution-phase combinatorial chemistry, Sun, (1999) *Comb Chem High Throughput Screen* 2(6):299-318, Recent advances in liquid-phase combinatorial chemistry); synthesis on soluble polymer (Gravert et al., (1997) *Curr Opin Chem Biol* 1(1):107-13, Synthesis on soluble polymers: new reactions and the construction of small molecules); and the like. See, e.g., Dolle et al., (1999) *J Comb Chem* 1(4):235-82, Comprehensive survey of cominatorial library synthesis: 1998. Freidinger RM., (1999) Nonpeptidic ligands for peptide and protein receptors, Current Opinion in Chemical Biology; and Kundu et al., *Prog Drug Res*;53:89-156, Combinatorial chemistry: polymer supported synthesis of peptide and non-peptide libraries). Compounds may be clinically tagged for ease of identification (Chabala, (1995) *Curr Opin Biotechnol* 6(6):633-9, Solid-phase combinatorial chemistry and novel tagging methods for identifying leads).

[0220] The combinatorial synthesis of carbohydrates and libraries containing oligosaccharides have been described (Schweizer et al., (1999) *Curr* Opin *Chem Biol* 3(3):291-8, Combinatorial synthesis of carbohydrates). The synthesis of natural-product based compound libraries has been described (Wessjohann, (2000) *Curr Opin Chem Biol* 4(3):303-9, Synthesis of natural-product based compound libraries).

[0221] Libraries of nucleic acids are prepared by various techniques, including by way of non-limiting example the ones described herein, for the isolation of aptamers. Libraries that include oligonucleotides and polyaminooligonucleotides (Markiewicz et al., (2000) Synthetic oligonucleotide combinatorial libraries and their applications, *Farmaco*. 55:174-7) displayed on streptavidin magnetic beads are known. Nucleic acid libraries are known

that can be coupled to parallel sampling and be deconvoluted without complex procedures such as automated mass spectrometry (Enjalbal C. Martinez J. Aubagnac JL, (2000) Mass spectrometry in combinatorial chemistry, *Mass Spectrometry Reviews*. 19:139-61) and parallel tagging. (Perrin DM., Nucleic acids for recognition and catalysis: landmarks, limitations, and looking to the future, *Combinatorial Chemistry & High Throughput Screening* 3:243-69).

[0222]   Peptidomimetics are identified using combinatorial chemistry and solid phase synthesis (Kim HO. Kahn M., (2000) A merger of rational drug design and combinatorial chemistry: development and application of peptide secondary structure mimetics, Combinatorial Chemistry & High Throughput Screening 3:167-83; al-Obeidi, (1998) *Mol Biotechnol* 9(3):205-23, Peptide and peptidomimetric libraries. Molecular diversity and drug design). The synthesis may be entirely random or based in part on a known polypeptide.

[0223]   Polypeptide libraries can be prepared according to various techniques. In brief, phage display techniques can be used to produce polypeptide ligands (Gram H., (1999) Phage display in proteolysis and signal transduction, Combinatorial Chemistry & High Throughput Screening. 2:19-28) that may be used as the basis for synthesis of peptidomimetics. Polypeptides, constrained peptides, proteins, protein domains, antibodies, single chain antibody fragments, antibody fragments, and antibody combining regions are displayed on filamentous phage for selection.

[0224]   Large libraries of individual variants of human single chain Fv antibodies have been produced. See, e.g., Siegel RW. Allen B. Pavlik P. Marks JD. Bradbury A., (2000) Mass spectral analysis of a protein complex using single-chain antibodies selected on a peptide target: applications to functional genomics, *Journal of Molecular Biology* 302:285-93; Poul MA. Becerril B. Nielsen UB. Morisson P. Marks JD.,(2000) Selection of tumor-specific internalizing human antibodies from phage libraries. Source *Journal of Molecular Biology*. 301:1149-61; Amersdorfer P. Marks JD., (2001) Phage libraries for generation of anti-botulinum scFv antibodies, *Methods in Molecular Biology*. 145:219-40; Hughes-Jones NC. Bye JM. Gorick BD. Marks JD. Ouwehand WH., (1999) Synthesis of Rh Fv phage-antibodies using VH and VL germline genes, *British Journal of Haematology*. 105:811-6; McCall AM. Amoroso AR. Sautes C. Marks JD. Weiner LM.,

(1998) Characterization of anti-mouse Fc gamma RII single-chain Fv fragments derived from human phage display libraries, *Immunotechnology*. 4:71-87; Sheets MD. Amersdorfer P. Finnern R. Sargent P. Lindquist E. Schier R. Hemingsen G. Wong C. Gerhart JC. Marks JD. Lindquist E., (1998) Efficient construction of a large nonimmune phage antibody library: the production of high-affinity human single-chain antibodies to protein antigens (published erratum appears in *Proc Natl Acad Sci* USA 1999 96:795), *Proc Natl Acad Sci USA* 95:6157-62).

[0225] Focused or smart chemical and pharmacophore libraries can be designed with the help of sophisticated strategies involving computational chemistry (e.g., Kundu B. Khare SK. Rastogi SK., (1999) Combinatorial chemistry: polymer supported synthesis of peptide and non-peptide libraries, *Progress in Drug Research* 53:89-156) and the use of structure-based ligands using database searching and docking, de novo drug design and estimation of ligand binding affinities (Joseph-McCarthy D., (1999) Computational approaches to structure-based ligand design, *Pharmacology & Therapeutics* 84:179-91; Kirkpatrick DL. Watson S. Ulhaq S., (1999) Structure-based drug design: combinatorial chemistry and molecular modeling, *Combinatorial Chemistry & High Throughput Screening*. 2:211-21; Eliseev AV. Lehn JM., (1999) Dynamic combinatorial chemistry: evolutionary formation and screening of molecular libraries, *Current Topics in Microbiology & Immunology* 243:159-72; Bolger et al., (1991) *Methods Enz.* 203:21-45; Martin, (1991) *Methods Enz.* 203:587-613; Neidle et al., (1991) *Methods Enz.* 203:433-458; U.S. Patent 6,178,384).

X. Crystallography

[0226] After binding compounds have been determined, the orientation of compound bound to target is determined. Preferably this determination involves crystallography on co-crystals of molecular scaffold compounds with target. Most protein crystallographic platforms can preferably be designed to analyze up to about 500 co-complexes of compounds, ligands, or molecular scaffolds bound to protein targets due to the physical parameters of the instruments and convenience of operation. If the number of scaffolds that have binding activity exceeds a number convenient for the application of crystallography methods, the scaffolds can be placed into groups based on having at least one common chemical structure or other desirable characteristics, and representative compounds can be selected from one or more of the classes. Classes can be made with

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increasingly exacting criteria until a desired number of classes (e.g., 500) is obtained. The classes can be based on chemical structure similarities between molecular scaffolds in the class, e.g., all possess a pyrrole ring, benzene ring, or other chemical feature. Likewise, classes can be based on shape characteristics, e.g., space-filling characteristics.

[0227] The co-crystallography analysis can be performed by co-complexing each scaffold with its target at concentrations of the scaffold that showed activity in the screening assay. This co-complexing can be accomplished with the use of low percentage organic solvents with the target molecule and then concentrating the target with each of the scaffolds. In preferred embodiments these solvents are less than 5% organic solvent such as dimethyl sulfoxide (DMSO), ethanol, methanol, or ethylene glycol in water or another aqueous solvent. Each scaffold complexed to the target molecule can then be screened with a suitable number of crystallization screening conditions at both 4 and 20 degrees. In preferred embodiments, about 96 crystallization screening conditions can be performed in order to obtain sufficient information about the co-complexation and crystallization conditions, and the orientation of the scaffold at the binding site of the target molecule. Crystal structures can then be analyzed to determine how the bound scaffold is oriented physically within the binding site or within one or more binding pockets of the molecular family member.

[0228] It is desirable to determine the atomic coordinates of the compounds bound to the target proteins in order to determine which is a most suitable scaffold for the protein family. X-ray crystallographic analysis is therefore most preferable for determining the atomic coordinates. Those compounds selected can be further tested with the application of medicinal chemistry. Compounds can be selected for medicinal chemistry testing based on their binding position in the target molecule. For example, when the compound binds at a binding site, the compound's binding position in the binding site of the target molecule can be considered with respect to the chemistry that can be performed on chemically tractable structures or sub-structures of the compound, and how such modifications on the compound might interact with structures or sub-structures on the binding site of the target. Thus, one can explore the binding site of the target and the chemistry of the scaffold in order to make decisions on how to modify the scaffold to arrive at a ligand with higher potency and/or selectivity. This process allows for more direct design of ligands, by utilizing structural and chemical information obtained directly

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from the co-complex, thereby enabling one to more efficiently and quickly design lead compounds that are likely to lead to beneficial drug products. In various embodiments it may be desirable to perform co-crystallography on all scaffolds that bind, or only those that bind with a particular affinity, for example, only those that bind with high affinity, moderate affinity, low affinity, very low affinity, or extremely low affinity. It may also be advantageous to perform co-crystallography on a selection of scaffolds that bind with any combination of affinities.

[0229] Standard X-ray protein diffraction studies such as by using a Rigaku RU-200® (Rigaku, Tokyo, Japan) with an X-ray imaging plate detector or a synchrotron beam-line can be performed on co-crystals and the diffraction data measured on a standard X-ray detector, such as a CCD detector or an X-ray imaging plate detector.

[0230] Performing X-ray crystallography on about 200 co-crystals should generally lead to about 50 co-crystals structures, which should provide about 10 scaffolds for validation in chemistry, which should finally result in about 5 selective leads for target molecules.

Virtual Assays

[0231] Commercially available software that generates three-dimensional graphical representations of the complexed target and compound from a set of coordinates provided can be used to illustrate and study how a compound is oriented when bound to a target. (e.g., QUANTA®, Accelerys, San Diego, CA). Thus, the existence of binding pockets at the binding site of the targets can be particularly useful in the present invention. These binding pockets are revealed by the crystallographic structure determination and show the precise chemical interactions involved in binding the compound to the binding site of the target. The person of ordinary skill will realize that the illustrations can also be used to decide where chemical groups might be added, substituted, modified, or deleted from the scaffold to enhance binding or another desirable effect, by considering where unoccupied space is located in the complex and which chemical substructures might have suitable size and/or charge characteristics to fill it. The person of ordinary skill will also realize that regions within the binding site can be flexible and its properties can change as a result of scaffold binding, and that chemical groups can be specifically targeted to those regions to achieve a desired effect. Specific locations on the molecular scaffold can be considered

with reference to where a suitable chemical substructure can be attached and in which conformation, and which site has the most advantageous chemistry available.

[0232] An understanding of the forces that bind the compounds to the target proteins reveals which compounds can most advantageously be used as scaffolds, and which properties can most effectively be manipulated in the design of ligands. The person of ordinary skill will realize that steric, ionic, hydrogen bond, and other forces can be considered for their contribution to the maintenance or enhancement of the target-compound complex. Additional data can be obtained with automated computational methods, such as docking and/or Free Energy Perturbations (FEP), to account for other energetic effects such as desolvation penalties. The compounds selected can be used to generate information about the chemical interactions with the target or for elucidating chemical modifications that can enhance selectivity of binding of the compound.

[0233] Computer models, such as homology models (i.e., based on a known, experimentally derived structure) can be constructed using data from the co-crystal structures. When the target molecule is a protein or enzyme, preferred co-crystal structures for making homology models contain high sequence identity in the binding site of the protein sequence being modeled, and the proteins will preferentially also be within the same class and/or fold family. Knowledge of conserved residues in active sites of a protein class can be used to select homology models that accurately represent the binding site. Homology models can also be used to map structural information from a surrogate protein where an apo or co-crystal structure exists to the target protein.

[0234] Virtual screening methods, such as docking, can also be used to predict the binding configuration and affinity of scaffolds, compounds, and/or combinatorial library members to homology models. Using this data, and carrying out "virtual experiments" using computer software can save substantial resources and allow the person of ordinary skill to make decisions about which compounds can be suitable scaffolds or ligands, without having to actually synthesize the ligand and perform co-crystallization. Decisions thus can be made about which compounds merit actual synthesis and co-crystallization. An understanding of such chemical interactions aids in the discovery and design of drugs that interact more advantageously with target proteins and/or are more selective for one

protein family member over others. Thus, applying these principles, compounds with superior properties can be discovered.

[0235] Additives that promote co-crystallization can of course be included in the target molecule formulation in order to enhance the formation of co-crystals. In the case of proteins or enzymes, the scaffold to be tested can be added to the protein formulation, which is preferably present at a concentration of approximately 1 mg/ml. The formulation can also contain between 0%-10% (v/v) organic solvent, e.g. DMSO, methanol, ethanol, propane diol, or 1,3 dimethyl propane diol (MPD) or some combination of those organic solvents. Compounds are preferably solubilized in the organic solvent at a concentration of about 10 mM and added to the protein sample at a concentration of about 100 mM. The protein-compound complex is then concentrated to a final concentration of protein of from about 5 to about 20 mg/ml. The complexation and concentration steps can conveniently be performed using a 96-well formatted concentration apparatus (e.g., Amicon Inc., Piscataway, NJ). Buffers and other reagents present in the formulation being crystallized can contain other components that promote crystallization or are compatible with crystallization conditions, such as DTT, propane diol, glycerol.

[0236] The crystallization experiment can be set-up by placing small aliquots of the concentrated protein-compound complex (1 µl) in a 96 well format and sampling under 96 crystallization conditions. (Other screening formats can also be used, e.g., plates with greater than 96 wells.) Crystals can typically be obtained using standard crystallization protocols that can involve the 96 well crystallization plate being placed at different temperatures. Co-crystallization varying factors other than temperature can also be considered for each protein-compound complex if desirable. For example, atmospheric pressure, the presence or absence of light or oxygen, a change in gravity, and many other variables can all be tested. The person of ordinary skill in the art will realize other variables that can advantageously be varied and considered.

Ligand Design and Preparation

[0237] The design and preparation of ligands can be performed with or without structural and/or co-crystallization data by considering the chemical structures in common between the active scaffolds of a set. In this process structure-activity hypotheses can be formed and those chemical structures found to be present in a substantial number of the

scaffolds, including those that bind with low affinity, can be presumed to have some effect on the binding of the scaffold. This binding can be presumed to induce a desired biochemical effect when it occurs in a biological system (e.g., a treated mammal). New or modified scaffolds or combinatorial libraries derived from scaffolds can be tested to disprove the maximum number of binding and/or structure-activity hypotheses. The remaining hypotheses can then be used to design ligands that achieve a desired binding and biochemical effect.

[0238] But in many cases it will be preferred to have co-crystallography data for consideration of how to modify the scaffold to achieve the desired binding effect (e.g., binding at higher affinity or with higher selectivity). Using the case of proteins and enzymes, co-crystallography data shows the binding pocket of the protein with the molecular scaffold bound to the binding site, and it will be apparent that a modification can be made to a chemically tractable group on the scaffold. For example, a small volume of space at a protein binding site or pocket might be filled by modifying the scaffold to include a small chemical group that fills the volume. Filling the void volume can be expected to result in a greater binding affinity, or the loss of undesirable binding to another member of the protein family. Similarly, the co-crystallography data may show that deletion of a chemical group on the scaffold may decrease a hindrance to binding and result in greater binding affinity or specificity.

[0239] It can be desirable to take advantage of the presence of a charged chemical group located at the binding site or pocket of the protein. For example, a positively charged group can be complemented with a negatively charged group introduced on the molecular scaffold. This can be expected to increase binding affinity or binding specificity, thereby resulting in a more desirable ligand. In many cases, regions of protein binding sites or pockets are known to vary from one family member to another based on the amino acid differences in those regions. Chemical additions in such regions can result in the creation or elimination of certain interactions (e.g., hydrophobic, electrostatic, or entropic) that allow a compound to be more specific for one protein target over another or to bind with greater affinity, thereby enabling one to synthesize a compound with greater selectivity or affinity for a particular family member. Additionally, certain regions can contain amino acids that are known to be more flexible than others. This often occurs in amino acids contained in loops connecting elements of the secondary structure of the protein, such as

alpha helices or beta strands. Additions of chemical moieties can also be directed to these flexible regions in order to increase the likelihood of a specific interaction occurring between the protein target of interest and the compound. Virtual screening methods can also be conducted *in silico* to assess the effect of chemical additions, subtractions, modifications, and/or substitutions on compounds with respect to members of a protein family or class.

[0240] The addition, subtraction, or modification of a chemical structure or sub-structure to a scaffold can be performed with any suitable chemical moiety. For example the following moieties, which are provided by way of example and are not intended to be limiting, can be utilized: hydrogen, alkyl, alkoxy, phenoxy, alkenyl, alkynyl, phenylalkyl, hydroxyalkyl, haloalkyl, aryl, arylalkyl, alkyloxy, alkylthio, alkenylthio, phenyl, phenylalkyl, phenylalkylthio, hydroxyalkyl-thio, alkylthiocarbbamylthio, cyclohexyl, pyridyl, piperidinyl, alkylamino, amino, nitro, mercapto, cyano, hydroxyl, a halogen atom, halomethyl, an oxygen atom (e.g., forming a ketone or N-oxide) or a sulphur atom (e.g., forming a thiol, thione, di-alkylsulfoxide or sulfone) are all examples of moieties that can be utilized.

[0241] Additional examples of structures or sub-structures that may be utilized are an aryl optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, carboxamide, nitro, and ester moieties; an amine of formula $-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and homocyclic or heterocyclic ring moieties; halogen or trihalomethyl; a ketone of formula $-COX_4$, where X_4 is selected from the group consisting of alkyl and homocyclic or heterocyclic ring moieties; a carboxylic acid of formula $-(X_5)_nCOOH$ or ester of formula $(X_6)_nCOOX_7$, where X_5, X_6, and X_7 and are independently selected from the group consisting of alkyl and homocyclic or heterocyclic ring moieties and where n is 0 or 1; an alcohol of formula $(X_8)_nOH$ or an alkoxy moiety of formula $-(X_8)_nOX_9$, where X_8 and X_9 are independently selected from the group consisting of saturated or unsaturated alkyl and homocyclic or heterocyclic ring moieties, wherein said ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, nitro, and ester and where n is 0 or 1; an amide of formula $NHCOX_{10}$, where X_{10} is selected from the group consisting of alkyl,

hydroxyl, and homocyclic or heterocyclic ring moieties, wherein said ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, nitro, and ester; SO_2, $NX_{11} X_{12}$, where X_{11} and X_{12} are selected from the group consisting of hydrogen, alkyl, and homocyclic or heterocyclic ring moieties; a homocyclic or heterocyclic ring moiety optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, carboxamide, nitro, and ester moieties; an aldehyde of formula -CHO; a sulfone of formula $-SO_2X_{13}$, where X_{13} is selected from the group consisting of saturated or unsaturated alkyl and homocyclic or heterocyclic ring moieties; and a nitro of formula $-NO_2$.

Identification of Attachment Sites on Molecular Scaffolds and Ligands

[0242]   In addition to the identification and development of ligands for phosphodiesterases and other enzymes, determination of the orientation of a molecular scaffold or other binding compound in a binding site allows identification of energetically allowed sites for attachment of the binding molecule to another component. For such sites, any free energy change associated with the presence of the attached component should not destablize the binding of the compound to the phosphodiesterase to an extent that will disrupt the binding. Preferably, the binding energy with the attachment should be at least 4 kcal/mol., more preferably at least 6, 8, 10, 12, 15, or 20 kcal/mol. Preferably, the presence of the attachment at the particular site reduces binding energy by no more than 3, 4, 5, 8, 10, 12, or 15 kcal/mol.

[0243]   In many cases, suitable attachment sites will be those that are exposed to solvent when the binding compound is bound in the binding site. In some cases, attachment sites can be used that will result in small displacements of a portion of the enzyme without an excessive energetic cost. Exposed sites can be identified in various ways. For example, exposed sites can be identified using a graphic display or 3-dimensional model. In a grahic display, such as a computer display, an image of a compound bound in a binding site can be visually inspected to reveal atoms or groups on the compound that are exposed to solvent and oriented such that attachment at such atom or group would not preclude binding of the enzyme and binding compound. Energetic costs of attachment can be calculated based on changes or distortions that would be caused by the attachment as well as entropic changes.

[0244] Many different types of components can be attached. Persons with skill are familiar with the chemistries used for various attachments. Examples of components that can be attached include, without limitation: solid phase components such as beads, plates, chips, and wells; a dlrect or indirect label; a linker, which may be a traceless linker; among others. Such linkers can themselves be attached to other components, *e.g.*, to solid phase media, labels, and/or binding moieties.

[0245] The binding energy of a compound and the effects on binding energy for attaching the molecule to another component can be calculated approximately using any of a variety of available software or by manual calculation. An example is the following:

[0246] Calculations were performed to estimate binding energies of different organic molecules to two Kinases: PIM-1 and CDK2. The organic molecules considered included Staurosporine, identified compounds that bind to PDE5A, and several linkers.

[0247] Calculated binding energies between protein-ligand complexes were obtained using the FlexX score (an implementation of the Bohm scoring function) within the Tripos software suite. The form for that equation is shown in the equation below:

$$\Delta G_{bind} = \Delta G_{tr} + \Delta G_{hb} + \Delta G_{ion} + \Delta G_{lipo} + \Delta G_{arom} + \Delta G_{rot}$$

[0248] where: ΔG_{tr} is a constant term that accounts for the overall loss of rotational and translational entropy of the lignand, ΔG_{hb} accounts for hydrogen bonds formed between the ligand and protein, ΔG_{ion} accounts for the ionic interactions between the ligand and protein, ΔG_{lipo} accounts for the lipophilic interaction that corresponds to the protein-ligand contact surface, ΔG_{arom} accounts for interactions between aromatic rings in the protein and ligand, and ΔG_{rot} accounts for the entropic penalty of restricting rotatable bonds in the ligand upon binding.

[0249] This method estimates the free energy that a lead compound should have to a target protein for which there is a crystal structure, and it accounts for the entropic penalty of flexible linkers. It can therefore be used to estimate the free energy penalty incurred by attaching linkers to molecules being screened and the binding energy that a lead compound should have in order to overcome the free energy penalty of the linker. The

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method does not account for solvation and the entropic penalty is likely overestimated for cases where the linker is bound to a solid phase through another binding complex, such as a biotin:streptavidin complex.

[0250] Co-crystals were aligned by superimposing residues of PIM-1 with corresponding residues in CDK2. The PIM-1 structure used for these calculations was a co-crystal of PIM-1 with a binding compound. The CDK2:Staurosporine co-crystal used was from the Brookhaven database file 1aq1. Hydrogen atoms were added to the proteins and atomic charges were assigned using the AMBER95 parameters within Sybyl. Modifications to the compounds described were made within the Sybyl modeling suite from Tripos.

[0251] These calcualtions indicate that the calculated binding energy for compounds that bind strongly to a given target (such as Staurosporine:CDK2) can be lower than –25 kcal/mol, while the calculated binding affinity for a good scaffold or an unoptimized binding compound can be in the range of -15 to -20. The free energy penalty for attachment to a linker such as the ethylene glycol or hexatriene is estimated as typically being in the range of +5 to +15 kcal/mol.

Linkers

[0252] Linkers suitable for use in the invention can be of many different types. Linkers can be selected for particular applications based on factors such as linker chemistry compatible for attachment to a binding compound and to another component utilized in the particular application. Additional factors can include, without limitation, linker length, linker stability, and ability to remove the linker at an appropriate time. Exemplary linkers include, but are not limited to, hexyl, hexatrienyl, ethylene glycol, and peptide linkers. Traceless linkers can also be used, *e.g.*, as described in Plunkett, M. J., and Ellman, J. A., (1995), *J. Org. Chem.*, 60:6006.

[0253] Typical functional groups, that are utilized to link binding compound(s), include, but not limited to, carboxylic acid, amine, hydroxyl, and thiol. (Examples can be found in Solid-supported combinatorial and parallel synthesis of small molecular weight compound libraries; (1998) Tetrahedron organic chemistry series Vol.17; Pergamon; p85).

Labels

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[0254] As indicated above, labels can also be attached to a binding compound or to a linker attached to a binding compound. Such attachment may be direct (attached directly to the binding compound) or indirect (attached to a component that is directly or indirectly attached to the binding compound). Such labels allow detection of the compound either directly or indirectly. Attachement of labels can be performed using conventional chemistries. Labels can include, for example, fluorescent labels, radiolabels, light scattering particles, light absorbent particles, magnetic particles, enzymes, and specific binding agents (*e.g.*, biotin or an antibody target moiety).

Solid Phase Media

[0255] Additional examples of components that can be attached directly or indirectly to a binding compound include various solid phase media. Similar to attachment of linkers and labels, attachment to solid phase media can be performed using conventional chemistries. Such solid phase media can include, for example, small components such as beads, nanoparticles, and fibers (*e.g.*, in suspension or in a gel or chromatographic matrix). Likewise, solid phase media can include larger objects such as plates, chips, slides, and tubes. In many cases, the binding compound will be attached in only a portion of such an objects, *e.g.*, in a spot or other local element on a generally flat surface or in a well or portion of a well.

Identification of Biological Agents

[0256] The posession of structural information about a protein also provides for the identification of useful biological agents, such as epitpose for development of antibodies, identification of mutation sites expected to affect activity, and identification of attachment sites allowing attachment of the protein to materials such as labels, linkers, peptides, and solid phase media.

[0257] Antibodies (Abs) finds multiple applications in a variety of areas including biotechnology, medicine and diagnosis, and indeed they are one of the most powerful tools for life science research. Abs directed against protein antigens can recognize either linear or native three-dimensional (3D) epitopes. The obtention of Abs that recognize 3D epitopes require the use of whole native protein (or of a portion that assumes a native conformation) as immunogens. Unfortunately, this not always a choice due to various technical reasons: for example the native protein is just not available, the protein is toxic,

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or its is desirable to utilize a high density antigen presentation. In such cases, immunization with peptides is the alternative. Of course, Abs generated in this manner will recognize linear epitopes, and they might or might not recognize the source native protein, but yet they will be useful for standard laboratory applications such as western blots. The selection of peptides to use as immunogens can be accomplished by following particular selection rules and/or use of epitope prediction software.

[0258] Though methods to predict antigenic peptides are not infallible, there are several rules that can be followed to determine what peptide fragments from a protein are likely to be antigenic. These rules are also dictated to increase the likelihood that an Ab to a particular peptide will recognize the native protein.

- 1. Antigenic peptides should be located in solvent accessible regions and contain both hydrophobic and hydrophilic residues.

  o For proteins of known 3D structure, solvent accessibility can be determined using a variety of programs such as DSSP, NACESS, or WHATIF, among others.

  o If the 3D structure is not known, use any of the following web servers to predict accessibilities: PHD, JPRED, PredAcc (c) ACCpro

- 2. Preferably select peptides lying in long loops connecting Secondary Structure (SS) motifs, avoiding peptides located in helical regions. This will increase the odds that the Ab recognizes the native protein. Such peptides can, for example, be identified from a crystal structure or crystal structure-based homology model.

  o For protein with known 3D coordinates, SS can be obtained from the sequence link of the relevant entry at the Brookhaven data bank. The PDBsum server also offer SS analysis of pdb records.

  o When no structure is available secondary structure predictions can be obtained from any of the following servers: PHD, JPRED, PSI-PRED, NNSP, etc

- 3. When possible, choose peptides that are in the N- and C-terminal region of the protein. Because the N- and C- terminal regions of proteins are usually solvent

accessible and unstructured, Abs against those regions are also likely to recognize the native protein.

- 4. For cell surface glycoproteins, eliminate from initial peptides those containing consesus sites for N-glycosilation.

    o N-glycosilation sites can be detected using Scanprosite, or NetNGlyc

[0259] In addition, several methods based on various physio-chemical properties of experimental determined epitopes (flexibility, hydrophibility, accessibility) have been published for the prediction of antigenic determinants and can be used. The antigenic index and Preditop are example.

[0260] Perhaps the simplest method for the prediction of antigenic determinants is that of Kolaskar and Tongaonkar, which is based on the occurrence of amino acid residues in experimentally determined epitopes. (Kolaskar and Tongaonkar (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBBS Lett.* 276(1-2):172-174.) The prediction algorithm works as follows:

- 1. Calculate the average propensity for each overlapping 7-mer and assign the result to the central residue (i+3) of the 7-mer.

- 2. Calculate the average for the whole protein.

- 3. (a) If the average for the whole protein is above 1.0 then all residues having average propensity above 1.0 are potentially antigenic.

- 3. (b) If the average for the whole protein is below 1.0 then all residues having above the average for the whole protein are potentially antigenic.

- 4. Find 8-mers where all residues are selected by step 3 above (6-mers in the original paper)

[0261] The Kolaskar and Tongaonkar method is also available from the GCG package, and it runs using the command *egcg*.

[0262] Crystal structures also allow identification of residues at which mutation is likely to alter the activity of the protein. Such residues include, for example, residues that

interact with susbtrate, conserved active site residues, and residues that are in a region of ordered secondary structure of involved in tertiary interactions. The mutations that are likely to affect activity will vary for different molecular contexts. Mutations in an active site that will affect activity are typically substitutions or deletions that eliminate a charge-charge or hydrogen bonding interaction, or introduce a steric interference. Mutations in secondary structure regions or molecular interaction regions that are likely to affect activity include, for example, substitutions that alter the hydrophobicity/hydrophilicity of a region, or that introduce a sufficient strain in a region near or including the active site so that critical residue(s) in the active site are displaced. Such substitutions and/or deletions and/or insertions are recognized, and the predicted structural and/or energetic effects of mutations can be calculated using conventional software.

IX. Phosphodiesterase Activity Assays

[0263] A number of different assays for phosphodiesterase activity can be utilized for assaying for active modulators and/or determining specificity of a modulator for a particular phosphodiesterase or group or phosphodiesterases. In addition to the assay mentioned in the Examples below, one of ordinary skill in the art will know of other assays that can be utilized and can modify an assay for a particular application. For example, numerous papers concerning PDE5 as well as papers concerning other PDEs described assays that can be used. For example, useful assays are described in Fryburg et al., U.S. Patent Application Publication 2002/0165237, Thompson et al., U.S. Patent Application Publication 2002/0009764, Pamukcu et al., U.S. Patent Application 09/046,739, and Pamukcu et al., U.S. Patent 6,500,610.

[0264] An assay for phosphodiesterase activity that can be used for PDE5A, can be performed according to the following procedure using purified PDE5A using the procedure described in Example 6.

[0265] Additional alternative assays can employ binding determinations. For example, this sort of assay can be formatted either in a fluorescence resonance energy transfer (FRET) format, or using an AlphaScreen (*a*mplified *l*uminescent *p*roximity *h*omogeneous *a*ssay) format by varying the donor and acceptor reagents that are attached to streptavidin or the phosphor-specific antibody.

X.   Organic Synthetic Techniques

[0266]   The versatility of computer-based modulator design and identification lies in the diversity of structures screened by the computer programs. The computer programs can search databases that contain very large numbers of molecules and can modify modulators already complexed with the enzyme with a wide variety of chemical functional groups. A consequence of this chemical diversity is that a potential modulator of phosphodiesterase function may take a chemical form that is not predictable. A wide array of organic synthetic techniques exist in the art to meet the challenge of constructing these potential modulators. Many of these organic synthetic methods are described in detail in standard reference sources utilized by those skilled in the art. One example of suh a reference is March, 1994, Advanced Organic Chemistry; Reactions, Mechanisms and Structure, New York, McGraw Hill. Thus, the techniques useful to synthesize a potential modulator of phosphodiesterase function identified by computer-based methods are readily available to those skilled in the art of organic chemical synthesis.

XI. Administration

[0267]   The methods and compounds will typically be used in therapy for human patients. However, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, sports animals, and pets such as horses, dogs and cats.

[0268]   Suitable dosage forms, in part, depend upon the use or the route of administration, for example, oral, transdermal, transmucosal, or by injection (parenteral). Such dosage forms should allow the compound to reach target cells. Other factors are well known in the art, and include considerations such as toxicity and dosage forms that retard the compound or composition from exerting its effects. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA, 1990 (hereby incorporated by reference herein).

[0269]   Compounds can be formulated as pharmaceutically acceptable salts. Pharmaceutically acceptable salts are non-toxic salts in the amounts and concentrations at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical characteristics of a compound without preventing it from exerting its physiological effect. Useful alterations in physical

properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate administering higher concentrations of the drug.

[0270] Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, chloride, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

[0271] Pharmaceutically acceptable salts also include basic addition salts such as those containing benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present. For example, see Remington's Pharmaceutical Sciences, 19th ed., Mack Publishing Co., Easton, PA, Vol. 2, p. 1457, 1995. Such salts can be prepared using the appropriate corresponding bases.

[0272] Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free-base form of a compound is dissolved in a suitable solvent, such as an aqueous or aqueous-alcohol in solution containing the appropriate acid and then isolated by evaporating the solution. In another example, a salt is prepared by reacting the free base and acid in an organic solvent.

[0273] The pharmaceutically acceptable salt of the different compounds may be present as a complex. Examples of complexes include 8-chlorotheophylline complex (analogous to, *e.g.*, dimenhydrinate: diphenhydramine 8-chlorotheophylline (1:1) complex; Dramamine) and various cyclodextrin inclusion complexes.

[0274] Carriers or excipients can be used to produce pharmaceutical compositions. The carriers or excipients can be chosen to facilitate administration of the compound. Examples of carriers include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable

oils, polyethylene glycols and physiologically compatible solvents. Examples of physiologically compatible solvents include sterile solutions of water for injection (WFI), saline solution, and dextrose.

[0275] The compounds can be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, transmucosal, rectal, or transdermal. Oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets, and liquid preparations such as syrups, elixirs, and concentrated drops.

[0276] Pharmaceutical preparations for oral use can be obtained, for example, by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross—linked polyvinylpyrrolidone, agar, or alginic acid, or a salt thereof such as sodium alginate.

[0277] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain, for example, gum arabic, talc, poly-vinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0278] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin ("gelcaps"), as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils,

liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

[0279] Alternatively, injection (parenteral administration) may be used, *e.g.,* intramuscular, intravenous, intraperitoneal, and/orsubcutaneous. For injection, the compounds of the invention are formulated in sterile liquid solutions, preferably in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

[0280] Administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays or suppositories (rectal or vaginal).

[0281] The amounts of various compound to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50}, the biological half-life of the compound, the age, size, and weight of the patient, and the disorder associated with the patient. The importance of these and other factors are well known to those of ordinary skill in the art. Generally, a dose will be between about 0.01 and 50 mg/kg, preferably 0.1 and 20 mg/kg of the patient being treated. Multiple doses may be used.

Manipulation of PDE5A

[0282] As the full-length coding sequence and amino acid sequence of PDE5A is known, cloning, construction of recombinant hPIM-3, production and purification of recombinant protein, introduction of PDE5A into other organisms, and other molecular biological manipulations of PDE5A are readily performed.

[0283] Techniques for the manipulation of nucleic acids, such as, e.g., subcloning, labeling probes (e.g., random-primer labeling using Klenow polymerase, nick translation,

amplification), sequencing, hybridization and the like are well disclosed in the scientific and patent literature, see, e.g., Sambrook, ed., Molecular Cloning: a Laboratory Manual (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); Current Protocols in Molecular Biology, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993). [0100] Nucleic acid sequences can be amplified as necessary for further use using amplification methods, such as PCR, isothermal methods, rolling circle methods, etc., are well known to the skilled artisan. See, e.g., Saiki, "Amplification of Genomic DNA" in PCR Protocols, Innis et al., Eds., Academic Press, San Diego, CA 1990, pp 13-20; Wharam et al., *Nucleic Acids Res.* 2001 Jun 1;29(11):E54-E54; Hafner et al., *Biotechniques* 2001 Apr;30(4):852-6, 858, 860 passim; Zhong et al., *Biotechniques* 2001 Apr;30(4):852-6, 858, 860 passim.

[0284] Nucleic acids, vectors, capsids, polypeptides, and the like can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, e.g., analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, e.g. fluid or gel precipitin reactions, immunodiffusion, immuno-electrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (e.g., SDS-PAGE), nucleic acid or target or signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography.

[0285] Obtaining and manipulating nucleic acids used to practice the methods of the invention can be performed by cloning from genomic samples, and, if desired, screening and re-cloning inserts isolated or amplified from, e.g., genomic clones or cDNA clones. Sources of nucleic acid used in the methods of the invention include genomic or cDNA libraries contained in, e.g., mammalian artificial chromosomes (MACs), see, e.g., U.S. Patent Nos. 5,721,118; 6,025,155; human artificial chromosomes, see, e.g., Rosenfeld (1997) *Nat. Genet.* 15:333-335; yeast artificial chromosomes (YAC); bacterial artificial

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chromosomes (BAC); P1 artificial chromosomes, see, e.g., Woon (1998) *Genomics* 50:306-316; P1-derived vectors (PACs), see, e.g., Kern (1997) *Biotechniques* 23:120-124; cosmids, recombinant viruses, phages or plasmids.

[0286] The nucleic acids of the invention can be operatively linked to a promoter. A promoter can be one motif or an array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter can include necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter which is active under most environmental and developmental conditions. An "inducible" promoter is a promoter which is under environmental or developmental regulation. A "tissue specific" promoter is active in certain tissue types of an organism, but not in other tissue types from the same organism. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0287] The nucleic acids of the invention can also be provided in expression vectors and cloning vehicles, e.g., sequences encoding the polypeptides of the invention. Expression vectors and cloning vehicles of the invention can comprise viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral DNA (e.g., vaccinia, adenovirus, foul pox virus, pseudorabies and derivatives of SV40), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as bacillus, *Aspergillus* and yeast). Vectors of the invention can include chromosomal, non-chromosomal and synthetic DNA sequences. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available.

[0288] The nucleic acids of the invention can be cloned, if desired, into any of a variety of vectors using routine molecular biological methods; methods for cloning *in vitro* amplified nucleic acids are disclosed, *e.g.*, U.S. Pat. No. 5,426,039. To facilitate cloning of amplified sequences, restriction enzyme sites can be "built into" a PCR primer pair.

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Vectors may be introduced into a genome or into the cytoplasm or a nucleus of a cell and expressed by a variety of conventional techniques, well described in the scientific and patent literature. See, e.g., Roberts (1987) *Nature* 328:731; Schneider (1995) *Protein Expr. Purif.* 6435:10; Sambrook, Tijssen or Ausubel. The vectors can be isolated from natural sources, obtained from such sources as ATCC or GenBank libraries, or prepared by synthetic or recombinant methods. For example, the nucleic acids of the invention can be expressed in expression cassettes, vectors or viruses which are stably or transiently expressed in cells (e.g., episomal expression systems). Selection markers can be incorporated into expression cassettes and vectors to confer a selectable phenotype on transformed cells and sequences. For example, selection markers can code for episomal maintenance and replication such that integration into the host genome is not required.

[0289] In one aspect, the nucleic acids of the invention are administered *in vivo* for *in situ* expression of the peptides or polypeptides of the invention. The nucleic acids can be administered as "naked DNA" (see, e.g., U.S. Patent No. 5,580,859) or in the form of an expression vector, e.g., a recombinant virus. The nucleic acids can be administered by any route, including peri- or intra-tumorally, as described below. Vectors administered *in vivo* can be derived from viral genomes, including recombinantly modified enveloped or non-enveloped DNA and RNA viruses, preferably selected from baculoviridiae, parvoviridiae, picornoviridiae, herpesveridiae, poxviridae, adenoviridiae, or picornnaviridiae. Chimeric vectors may also be employed which exploit advantageous merits of each of the parent vector properties (See e.g., Feng (1997) *Nature Biotechnology* 15:866-870). Such viral genomes may be modified by recombinant DNA techniques to include the nucleic acids of the invention; and may be further engineered to be replication deficient, conditionally replicating or replication competent. In alternative aspects, vectors are derived from the adenoviral (e.g., replication incompetent vectors derived from the human adenovirus genome, see, e.g., U.S. Patent Nos. 6,096,718; 6,110,458; 6,113,913; 5,631,236); adeno-associated viral and retroviral genomes. Retroviral vectors can include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof; see, e.g., U.S. Patent Nos. 6,117,681; 6,107,478; 5,658,775; 5,449,614; Buchscher (1992) J. Virol. 66:2731-2739; Johann (1992) *J. Virol.* 66:1635-1640). Adeno-associated virus (AAV)-based vectors can be used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and peptides, and in *in*

vivo and *ex vivo* gene therapy procedures; see, e.g., U.S. Patent Nos. 6,110,456; 5,474,935; Okada (1996) *Gene Ther.* 3:957-964.

[0290] The present invention also relates to fusion proteins, and nucleic acids encoding them. A polypeptide of the invention can be fused to a heterologous peptide or polypeptide, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification. Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, e.g., producing a more immunogenic peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, e.g., metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between a purification domain and the motif-comprising peptide or polypeptide to facilitate purification. For example, an expression vector can include an epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see e.g., Williams (1995) *Biochemistry* 34:1787-1797; Dobeli (1998) *Protein Expr. Purif.* 12:404-414). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. In one aspect, a nucleic acid encoding a polypeptide of the invention is assembled in appropriate phase with a leader sequence capable of directing secretion of the translated polypeptide or fragment thereof. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well disclosed in the scientific and patent literature, see e.g., Kroll (1993) *DNA Cell. Biol.* 12:441-53.

[0291] The nucleic acids and polypeptides of the invention can be bound to a solid support, e.g., for use in screening and diagnostic methods. Solid supports can include, e.g., membranes (e.g., nitrocellulose or nylon), a microtiter dish (e.g., PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dip stick (e.g., glass, PVC, polypropylene, polystyrene, latex and the like), a microfuge tube, or a glass, silica, plastic,

metallic or polymer bead or other substrate such as paper. One solid support uses a metal (e.g., cobalt or nickel)-comprising column which binds with specificity to a histidine tag engineered onto a peptide.

[0292] Adhesion of molecules to a solid support can be direct (i.e., the molecule contacts the solid support) or indirect (a "linker" is bound to the support and the molecule of interest binds to this linker). Molecules can be immobilized either covalently (e.g., utilizing single reactive thiol groups of cysteine residues (see, e.g., Colliuod (1993) *Bioconjugate Chem.* 4:528-536) or non-covalently but specifically (e.g., via immobilized antibodies (see, e.g., Schuhmann (1991) *Adv. Mater.* 3:388-391; Lu (1995) *Anal. Chem.* 67:83-87; the biotin/strepavidin system (see, e.g., Iwane (1997) *Biophys. Biochem. Res. Comm.* 230:76-80); metal chelating, e.g., Langmuir-Blodgett films (see, e.g., Ng (1995) *Langmuir* 11:4048-55); metal-chelating self-assembled monolayers (see, e.g., Sigal (1996) *Anal. Chem.* 68:490-497) for binding of polyhistidine fusions.

[0293] Indirect binding can be achieved using a variety of linkers which are commercially available. The reactive ends can be any of a variety of functionalities including, but not limited to: amino reacting ends such as N-hydroxysuccinimide (NHS) active esters, imidoesters, aldehydes, epoxides, sulfonyl halides, isocyanate, isothiocyanate, and nitroaryl halides; and thiol reacting ends such as pyridyl disulfides, maleimides, thiophthalimides, and active halogens. The heterobifunctional crosslinking reagents have two different reactive ends, e.g., an amino-reactive end and a thiol-reactive end, while homobifunctional reagents have two similar reactive ends, e.g., bismaleimidohexane (BMH) which permits the cross-linking of sulfhydryl-containing compounds. The spacer can be of varying length and be aliphatic or aromatic. Examples of commercially available homobifunctional cross-linking reagents include, but are not limited to, the imidoesters such as dimethyl adipimidate dihydrochloride (DMA); dimethyl pimelimidate dihydrochloride (DMP); and dimethyl suberimidate dihydrochloride (DMS). Heterobifunctional reagents include commercially available active halogen-NHS active esters coupling agents such as N-succinimidyl bromoacetate and N-succinimidyl (4-iodoacetyl)aminobenzoate (SIAB) and the sulfosuccinimidyl derivatives such as sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-SIAB) (Pierce). Another group of coupling agents is the heterobifunctional and thiol cleavable agents

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such as N-succinimidyl 3-(2-pyridyidithio)propionate (SPDP) (Pierce Chemicals, Rockford, IL).

[0294] Antibodies can also be used for binding polypeptides and peptides of the invention to a solid support. This can be done directly by binding peptide-specific antibodies to the column or it can be done by creating fusion protein chimeras comprising motif-containing peptides linked to, e.g., a known epitope (e.g., a tag (e.g., FLAG, myc) or an appropriate immunoglobulin constant domain sequence (an "immunoadhesin," see, e.g., Capon (1989) *Nature* 377:525-531 (1989).

[0295] Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. For example, in one aspect of the invention, a monitored parameter is transcript expression of a gene comprising a nucleic acid of the invention. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array, or "biochip." By using an "array" of nucleic acids on a microchip, some or all of the transcripts of a cell can be simultaneously quantified. Alternatively, arrays comprising genomic nucleic acid can also be used to determine the genotype of a newly engineered strain made by the methods of the invention. Polypeptide arrays" can also be used to simultaneously quantify a plurality of proteins.

[0296] The terms "array" or "microarray" or "biochip" or "chip" as used herein is a plurality of target elements, each target element comprising a defined amount of one or more polypeptides (including antibodies) or nucleic acids immobilized onto a defined area of a substrate surface. In practicing the methods of the invention, any known array and/or method of making and using arrays can be incorporated in whole or in part, or variations thereof, as disclosed, for example, in U.S. Patent Nos. 6,277,628; 6,277,489; 6,261,776; 6,258,606; 6,054,270; 6,048,695; 6,045,996; 6,022,963; 6,013,440; 5,965,452; 5,959,098; 5,856,174; 5,830,645; 5,770,456; 5,632,957; 5,556,752; 5,143,854; 5,807,522; 5,800,992; 5,744,305; 5,700,637; 5,556,752; 5,434,049; see also, e.g., WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; see also, e.g., Johnston (1998) *Curr. Biol.* 8:R171-R174;

Schummer (1997) *Biotechniques* 23:1087-1092; Kern (1997) *Biotechniques* 23:120-124; Solinas-Toldo (1997) *Genes, Chromosomes & Cancer* 20:399-407; Bowtell (1999) *Nature Genetics Supp.* 21:25-32. See also published U.S. patent applications Nos. 20010018642; 20010019827; 20010016322; 20010014449; 20010014448; 20010012537; 20010008765.

Host Cells and Transformed Cells

[0297]   The invention also provides a transformed cell comprising a nucleic acid sequence of the invention, *e.g.*, a sequence encoding a polypeptide of the invention, or a vector of the invention. The host cell may be any of the host cells familiar to those skilled in the art, including prokaryotic cells, eukaryotic cells, such as bacterial cells, fungal cells, yeast cells, mammalian cells, insect cells, or plant cells. Exemplary bacterial cells include *E. coli*, *Streptomyces*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. Exemplary insect cells include *Drosophila* S2 and *Spodoptera* Sf9. Exemplary animal cells include CHO, COS or Bowes melanoma or any mouse or human cell line. The selection of an appropriate host is within the abilities of those skilled in the art.

[0298]   Vectors may be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation.

[0299]   Engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the invention. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be induced by appropriate means (e.g., temperature shift or chemical induction) and the cells may be cultured for an additional period to allow them to produce the desired polypeptide or fragment thereof.

[0300]   Cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract is retained for further purification. Microbial cells employed for expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art. The expressed polypeptide or fragment

can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. If desired, high performance liquid chromatography (HPLC) can be employed for final purification steps.

[0301] Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts and other cell lines capable of expressing proteins from a compatible vector, such as the C127, 3T3, CHO, HeLa and BHK cell lines.

[0302] The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Depending upon the host employed in a recombinant production procedure, the polypeptides produced by host cells containing the vector may be glycosylated or may be non-glycosylated. Polypeptides of the invention may or may not also include an initial methionine amino acid residue.

[0303] Cell-free translation systems can also be employed to produce a polypeptide of the invention. Cell-free translation systems can use mRNAs transcribed from a DNA construct comprising a promoter operably linked to a nucleic acid encoding the polypeptide or fragment thereof. In some aspects, the DNA construct may be linearized prior to conducting an *in vitro* transcription reaction. The transcribed mRNA is then incubated with an appropriate cell-free translation extract, such as a rabbit reticulocyte extract, to produce the desired polypeptide or fragment thereof.

[0304] The expression vectors can contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

[0305] For transient expression in mammalian cells, cDNA encoding a polypeptide of interest may be incorporated into a mammalian expression vector, e.g. pcDNA1, which is available commercially from Invitrogen Corporation (San Diego, Calif., U.S.A.; catalogue

number V490-20). This is a multifunctional 4.2 kb plasmid vector designed for cDNA expression in eukaryotic systems, and cDNA analysis in prokaryotes, incorporated on the vector are the CMV promoter and enhancer, splice segment and polyadenylation signal, an SV40 and Polyoma virus origin of replication, and M13 origin to rescue single strand DNA for sequencing and mutagenesis, Sp6 and T7 RNA promoters for the production of sense and anti-sense RNA transcripts and a Col E1-like high copy plasmid origin. A polylinker is located appropriately downstream of the CMV promoter (and 3' of the T7 promoter).

[0306] The cDNA insert may be first released from the above phagemid incorporated at appropriate restriction sites in the pcDNAI polylinker. Sequencing across the junctions may be performed to confirm proper insert orientation in pcDNAI. The resulting plasmid may then be introduced for transient expression into a selected mammalian cell host, for example, the monkey-derived, fibroblast like cells of the COS-1 lineage (available from the American Type Culture Collection, Rockville, Md. as ATCC CRL 1650).

[0307] For transient expression of the protein-encoding DNA, for example, COS-1 cells may be transfected with approximately 8 µg DNA per 10^6 COS cells, by DEAE-mediated DNA transfection and treated with chloroquine according to the procedures described by Sambrook et al, Molecular Cloning: A Laboratory Manual, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y, pp. 16.30-16.37. An exemplary method is as follows. Briefly, COS-1 cells are plated at a density of 5 x 10^6 cells/dish and then grown for 24 hours in FBS-supplemented DMEM/F12 medium. Medium is then removed and cells are washed in PBS and then in medium. A transfection solution containing DEAE dextran (0.4 mg/ml), 100 µM chloroquine, 10% NuSerum, DNA (0.4 mg/ml) in DMEM/F12 medium is then applied on the cells 10 ml volume. After incubation for 3 hours at 37 °C, cells are washed in PBS and medium as just described and then shocked for 1 minute with 10% DMSO in DMEM/F12 medium. Cells are allowed to grow for 2-3 days in 10% FBS-supplemented medium, and at the end of incubation dishes are placed on ice, washed with ice cold PBS and then removed by scraping. Cells are then harvested by centrifugation at 1000 rpm for 10 minutes and the cellular pellet is frozen in liquid nitrogen, for subsequent use in protein expression. Northern blot analysis of a thawed aliquot of frozen cells may be used to confirm expression of receptor-encoding cDNA in cells under storage.

[0308] In a like manner, stably transfected cell lines can also prepared, for example, using two different cell types as host: CHO K1 and CHO Pro5. To construct these cell lines, cDNA coding for the relevant protein may be incorporated into the mammalian expression vector pRC/CMV (Invitrogen), which enables stable expression. Insertion at this site places the cDNA under the expression control of the cytomegalovirus promoter and upstream of the polyadenylation site and terminator of the bovine growth hormone gene, and into a vector background comprising the neomycin resistance gene (driven by the SV40 early promoter) as selectable marker.

[0309] An exemplary protocol to introduce plasmids constructed as described above is as follows. The host CHO cells are first seeded at a density of 5×10^5 in 10% FBS-supplemented MEM medium. After growth for 24 hours, fresh medium is added to the plates and three hours later, the cells are transfected using the calcium phosphate-DNA co-precipitation procedure (Sambrook et al, supra). Briefly, 3 μg of DNA is mixed and incubated with buffered calcium solution for 10 minutes at room temperature. An equal volume of buffered phosphate solution is added and the suspension is incubated for 15 minutes at room temperature. Next, the incubated suspension is applied to the cells for 4 hours, removed and cells were shocked with medium containing 15% glycerol. Three minutes later, cells are washed with medium and incubated for 24 hours at normal growth conditions. Cells resistant to neomycin are selected in 10% FBS-supplemented alpha-MEM medium containing G418 (1 mg/ml). Individual colonies of G418-resistant cells are isolated about 2-3 weeks later, clonally selected and then propagated for assay purposes.

EXAMPLES

[0310] A number of examples involved in the present invention are described below. In most cases, alternative techniques could also be used. For example, techniques, methods, and other information described in Whitaker et al., U.S. Patent Application 2001/0053780 can be used in the present invention. Such techniques and information include, without limitation, cloning, culturing, purification, assaying, screening, use of modulators, sequence information, and information concerning biological role of PDE5A. Each of these references is incorporated by reference herein in its entirety, including drawings.

EXAMPLE 1: Cloning of PDE5A Phosphodiesterase Domain

[0311] PDE5A cDNA sequence was amplified from a Human Kidney QUICK-Clone cDNA library (Clontech, #7112-1) by PCR using the following primers:

PDE5A-S: 5'-GTCGTAT CATATG TCAGCAGCAGAGGAAGAAAC-3' 33 mer
PDE5A-A: 5'-TCTGCA GTCGAC AGGCCACTCAGTTCCGCTTG-3' 32 mer

[0312] The resulting PCR fragment was digested with NdeI and SalI and subcloned into the pET15S vector (shown below). In this expression plasmid, residues 531-875 of PDE5A are in frame with an N-terminal His-tag followed by a thrombin cleavage site.

[0313] The sequence of pET15S, with multi-cloning site is shown below:

T7 promoter

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCC

RBS
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACC

                                                   NdeI
ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGC**CATATG**GGATCCGG

 M G S S H H H H H H S S G L V P R G S H M --------

        StuI   **SalI**
AATTCAAAGGCCTAC**GTCGAC**TAGAGCCTGCAGT*CTCGAC*CATCATCATCATCATTAATAAAAGG▮

-------------------- *

              SpeI   **BamHI**
▮GGCCGTT**ACTAGT**GGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGG

 IVEX-3 Primer

      Bpu1102 I   **T7 terminator**
CTGCTGCCACC▮ACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
       3'-PET Primer

[0314] pET15S vector is derived from pET15b vector (Novagen) for bacterial expression to produce the proteins with N-terminal His6. This vector was modified by

replacement of NdeI-BamHI fragment to others to create a SalI site and stop codon (TAG). Vector size is 5814 bp. Insertion can be performed using NdeI-SalI site.

[0315] The nucleotide and amino acid sequences for the PDE5A phosphodiesterase domain utilized encompass amino acids 531-875 of the amino acid sequence provided in Table 4.

EXAMPLE 2:   Expression and Purification of PDE5A Phosphodiesterase Domain

[0316] PDE 5A is purified from *E. coli* cells [BL21(DE3)Codon Plus(RIL) (Novagen)] grown in Terrific broth that has been supplemented with 0.2mM Zinc Acetate and 1mM MgCl2 and induced for 16-20h with 1 mM IPTG at 22 C. The centrifuged bacterial pellet (typically 200-250g from 16 L) is suspended in lysis buffer (0.1M potassium phosphate buffer, pH 8.0, 10% glycerol, 1 mM PMSF). 100ug/ml of lysozyme is added to the lysate and the cells are lysed in a Cell Disruptor (MircoFluidics). The cell extract is clarified at 5000 rpm in a Sorvall SA6000 rotor for 1h, and the supernatant is recentrifuged for another hour at 17000 rpm in a Sorvall SA 600 rotor. 5 mM imidazole (pH 8.0) is added to the clarified supernatant and 2 ml of cobalt beads (50% slurry) is added to each 35 ml of extract. The beads are mixed at 4 C for 3-4 h on a Nutator and the beads are recovered by centrifugation at 4000 rpm for 3 min. The pelleted beads are washed several times with lysis buffer and the beads are packed on a BioRad disposable column. The bound protein is eluted with 3-4 column volumes of 0.1M imidazole followed by 0.25M imidazole, both prepared in lysis buffer. The protein eluted from the cobalt beads is concentrated on Centriprep-10 membranes (Amicon) and separated on a Pharmacia Superdex 200 column (26/60) in low salt buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM beta-mercaptoethanol). The uncleaved PDE5A is purified by hydroxyapatite chromatography eluted with a phosphate gradient. A final buffer exchange is done on a Pharmacia Superdex 200 column (26/60) in 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM beta-mercaptoethanol.

Example 3: Crystallization of PDE5A Phosphodiesterase Domain

[0317] Crystals of purified PDE5 were grown in 10% (w/v) PEG3000, 100 mM phosphate-citrate (pH 4.3), 200 mM NaCl, 1mM DTT, 1mM Sp-cAMP and 8 mg/ml

protein at 4°C, using an Intelliplate (Robbins Scientific, Hampton) by mixing one microliter of protein with one microliter of precipitant, also at 4°C.

Example 4: Diffraction Analysis of PDE5A

[0318] Synchrotron X-ray data for PDE5A was collected at beamline 8.3.1 of the Advanced Light Source (ALS, Lawrence Berkeley National Laboratory, Berkeley) on a Quantum 210 charge-coupled device detector ($\lambda = 1.10$Å). The data were processed using Mosflm () and scaled and reduced with Scala () in CCP4 (). The data processing process was driven by the ELVES automation scripts.

[0319] A ribbon diagram of the PDE5A catalytic domain is shown in Figure 1. Atomic coordinates for the apo protein are provided in Table 1.

Example 5: PDE5A Binding Assays

[0320] Binding assays can be performed in a variety of ways, including a variety of ways known in the art. For example, as indicated above, binding assays can be performed using fluorescence resonance energy transfer (FRET) format, or using an AlphaScreen

[0321] Alternatively, any method which can measure binding of a ligand to the cGMP-binding site can be used. For example, a fluorescent ligand can be used. When bound to PDE5A, the emitted fluorescence is polarized. Once displaced by inhibitor binding, the polarization decreases.

[0322] Determination of IC50 for compounds by competitive binding assays. (Note that K_I is the dissociation constant for inhibitor binding; K_D is the dissociation constant for substrate binding.) For this system, the IC50, inhibitor binding constant and substrate binding constant can be interrelated according to the following formula:

[0323] When using radiolabeled substrate $K_I = \dfrac{IC50}{1 + [L^*]/K_D}$,

[0324] the IC50 ~ K_I when there is a small amount of labeled substrate.

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Example 6: PDE5A Activity Assay

[0325] As an exemplary phosphodiesterase assay, the effect of potential modulators phosphodiesterase activity of PDE5A and other PDEs was measured in the following assay format:

Reagents

Assay Buffer

      50 mM Tris, 7.5

      8.3 mM $MgCl_2$

      1.7 mM EGTA

      0.01% BSA

      Store @ 4 degrees

RNA binding YSi SPA beads

Beads are 100 mg/ml in water. Dilute to 5 mg/ml in 18 mM Zn using 1M ZnAcetate/$ZnSO_4$ solution(3:1) and water. Store @ 4 degrees.

Low control compounds	Concentration of 20X DMSO Stock
PDE1B: 8-methoxymethyl IBMX	20 mM
PDE2A: EHNA	10 mM
PDE3B: Milrinone	2 mM
PDE4D: Rolipram	10 mM
PDE5A: Zaprinast	10 mM
PDE7B: IBMX	40 mM
PDE10A: Dipyridamole	4 mM

Enzyme concentrations (2X final concentration. Diluted in assay buffer)

PDE1B 50 ng/ml

PDE2A 50 ng/ml

PDE3B 10 ng/ml

PDE4D 5 ng/ml

PDE5A 20 ng/ml

PDE7B 25 ng/ml

PDE10A 5 ng/ml)

Radioligands

[^{3}H] cAMP (Amersham TRK559). Dilute 2000X in assay buffer.

[^{3}H] cGMP (Amersham TRK392). For PDE5A assay only. Dilute 2000X in assay buffer.

Protocol

- Make assay plates from 2mM, 96 well master plates by transferring 1ul of
- compound to 384 well plate using BiomekFx. Final concentration of compounds will be ~100 μM. Duplicate assay plates are prepared from each master plate so that compounds are assayed in duplicate.
- To column 23 of the assay plate add 1ul of 20X DMSO stock of appropriate control compound. These will be the low controls.
- Columns 1 and 2 of Chembridge library assay plates and columns 21 and 22 of the Maybridge library assay plates have 1ul DMSO. These are the high controls.
- Using BiomekFx, pipet 10 μl of radioligand into each assay well, then, using the same tips, pipet 10 μl of enzyme into each well.
- Seal assay plate with transparent cover. Centrifuge briefly @ 1000 RPM, them mix on plate shaker for 10 s.
- Incubate @ 30° for 30 min.
- Using BiomekFx, add 10 μl of bead mixture to each assay well. Mix beads thoroughly in reservoir immediately prior to each assay plate addition.
- Re-seal plate with fresh transparent cover. Mix on plate shaker for 10 s, then centrifuge for 1 min. @ 1000 RPM.
- Place plates in counting racks. Let stand for ≥ 30 min, then count on Wallac TriLux using program 8.
- Analyze data as % inhibition of enzyme activity. Average of high controls = 0% inhibition. Average of low controls = 100% inhibition.

Example 9: Site-directed Mutagenesis of PDE5A

[0326] Mutagenesis of PDE5A can be carried out according to the following procedure as described in Molecular Biology: Current Innovations and Future Trends. Eds. A.M. Griffin and H.G.Griffin. (1995) ISBN 1-898486-01-8, Horizon Scientific Press, PO Box 1, Wymondham, Norfolk, U.K., among others.

[0327] In vitro site-directed mutagenesis is an invaluable technique for studying protein structure-function relationships, gene expression and vector modification. Several methods have appeared in the literature, but many of these methods require single-stranded DNA as the template. The reason for this, historically, has been the need for separating the complementary strands to prevent reannealing. Use of PCR in site-directed mutagenesis accomplishes strand separation by using a denaturing step to separate the complementing strands and allowing efficient polymerization of the PCR primers. PCR site-directed methods thus allow site-specific mutations to be incorporated in virtually any double-stranded plasmid; eliminating the need for M13-based vectors or single-stranded rescue.

[0328] It is often desirable to reduce the number of cycles during PCR when performing PCR-based site-directed mutagenesis to prevent clonal expansion of any (undesired) second-site mutations. Limited cycling which would result in reduced product yield, is offset by increasing the starting template concentration. A selection is used to reduce the number of parental molecules coming through the reaction. Also, in order to use a single PCR primer set, it is desirable to optimize the long PCR method. Further, because of the extendase activity of some thermostable polymerases it is often necessary to incorporate an end-polishing step into the procedure prior to end-to-end ligation of the PCR-generated product containing the incorporated mutations in one or both PCR primers.

[0329] The following protocol provides a facile method for site-directed mutagenesis and accomplishes the above desired features by the incorporation of the following steps: (i) increasing template concentration approximately 1000-fold over conventional PCR conditions; (ii) reducing the number of cycles from 25-30 to 5-10; (iii) adding the restriction endonuclease DpnI (recognition target sequence: 5-Gm6ATC-3, where the A residue is methylated) to select against parental DNA (note: DNA isolated from almost all common strains of E. coli is Dam-methylated at the sequence 5-GATC-3); (iv) using Taq Extender in the PCR mix for increased reliability for PCR to 10 kb; (v) using Pfu DNA

polymerase to polish the ends of the PCR product, and (vi) efficient intramolecular ligation in the presence of T4 DNA ligase.

[0330] Plasmid template DNA (approximately 0.5 pmole) is added to a PCR cocktail containing, in 25 ul of 1x mutagenesis buffer: (20 mM Tris HCl, pH 7.5; 8 mM MgCl2; 40 ug/ml BSA); 12-20 pmole of each primer (one of which must contain a 5-prime phosphate), 250 uM each dNTP, 2.5 U Taq DNA polymerase, 2.5 U of Taq Extender (Stratagene).

[0331] The PCR cycling parameters are 1 cycle of: 4 min at 94 C, 2 min at 50 C and 2 min at 72 C; followed by 5-10 cycles of 1 min at 94 C, 2 min at 54 C and 1 min at 72 C (step 1).

[0332] The parental template DNA and the linear, mutagenesis-primer incorporating newly synthesized DNA are treated with DpnI (10 U) and Pfu DNA polymerase (2.5U). This results in the DpnI digestion of the in vivo methylated parental template and hybrid DNA and the removal, by Pfu DNA polymerase, of the Taq DNA polymerase-extended base(s) on the linear PCR product.

[0333] The reaction is incubated at 37 C for 30 min and then transferred to 72 C for an additional 30 min (step 2).

[0334] Mutagenesis buffer (1x, 115 ul, containing 0.5 mM ATP) is added to the DpnI-digested, Pfu DNA polymerase-polished PCR products.

[0335] The solution is mixed and 10 ul is removed to a new microfuge tube and T4 DNA ligase (2-4 U) added.

[0336] The ligation is incubated for greater than 60 min at 37 C (step 3).

[0337] The treated solution is transformed into competent E. coli (step 4).

[0338] In addition to the PCR-based site-directed mutagenesis described above, other methods are available. Examples include those described in Kunkel (1985) Proc. Natl. Acad. Sci. 82:488-492; Eckstein et al. (1985) Nucl. Acids Res. 13:8764-8785; and using the GeneEditor™ Site-Directed Mutageneis Sytem from Promega.

[0339] All patents and other references cited in the specification are indicative of the level of skill of those skilled in the art to which the invention pertains, and are incorporated by reference in their entireties, including any tables and figures, to the same extent as if each reference had been incorporated by reference in its entirety individually.

[0340] One skilled in the art would readily appreciate that the present invention is well adapted to obtain the ends and advantages mentioned, as well as those inherent therein. The methods, variances, and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

[0341] It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. For example, variations can be made to crystallization or co-crystallization conditions for PDE5A proteins and/or various phosphodiesterase domain sequences can be used. Thus, such additional embodiments are within the scope of the present invention and the following claims.

[0342] The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0343] In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

[0344] Also, unless indicated to the contrary, where various numerical values are provided for embodiments, additional embodiments are described by taking any 2 different specified values as the endpoints of a range. Such ranges are also within the scope of the present described invention.

[0345] Thus, additional embodiments are within the scope of the invention and within the following claims.

Table 1

```
HEADER        ----                        XX-XXX-XX    xxxx
COMPND        ---
REMARK    3
REMARK    3 REFINEMENT.
REMARK    3    PROGRAM      : REFMAC 5.1.25
REMARK    3    AUTHORS      : MURSHUDOV,VAGIN,DODSON
REMARK    3
REMARK    3    REFINEMENT TARGET : MAXIMUM LIKELIHOOD
REMARK    3
REMARK    3 DATA USED IN REFINEMENT.
REMARK    3    RESOLUTION RANGE HIGH (ANGSTROMS) :   2.10
REMARK    3    RESOLUTION RANGE LOW  (ANGSTROMS) :  84.51
REMARK    3    DATA CUTOFF            (SIGMA(F)) : NONE
REMARK    3    COMPLETENESS FOR RANGE        (%) :  99.35
REMARK    3    NUMBER OF REFLECTIONS             :   23081
REMARK    3
REMARK    3 FIT TO DATA USED IN REFINEMENT.
REMARK    3    CROSS-VALIDATION METHOD          : THROUGHOUT
REMARK    3    FREE R VALUE TEST SET SELECTION  : RANDOM
REMARK    3    R VALUE      (WORKING + TEST SET) : 0.20593
REMARK    3    R VALUE            (WORKING SET) : 0.20404
REMARK    3    FREE R VALUE                     : 0.24234
REMARK    3    FREE R VALUE TEST SET SIZE   (%) : 5.0
REMARK    3    FREE R VALUE TEST SET COUNT      : 1227
REMARK    3
REMARK    3 FIT IN THE HIGHEST RESOLUTION BIN.
REMARK    3    TOTAL NUMBER OF BINS USED        :      20
REMARK    3    BIN RESOLUTION RANGE HIGH        :    2.100
REMARK    3    BIN RESOLUTION RANGE LOW         :    2.155
REMARK    3    REFLECTION IN BIN     (WORKING SET) :    1696
REMARK    3    BIN R VALUE          (WORKING SET) :   0.296
REMARK    3    BIN FREE R VALUE SET COUNT       :      84
REMARK    3    BIN FREE R VALUE                 :   0.336
REMARK    3
REMARK    3 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
REMARK    3    ALL ATOMS               :    2555
REMARK    3
REMARK    3 B VALUES.
REMARK    3    FROM WILSON PLOT          (A**2) : NULL
REMARK    3    MEAN B VALUE       (OVERALL, A**2) :  32.944
REMARK    3    OVERALL ANISOTROPIC B VALUE.
REMARK    3     B11 (A**2) :    -1.34
REMARK    3     B22 (A**2) :    -1.34
REMARK    3     B33 (A**2) :     2.01
REMARK    3     B12 (A**2) :    -0.67
REMARK    3     B13 (A**2) :     0.00
REMARK    3     B23 (A**2) :     0.00
REMARK    3
REMARK    3 ESTIMATED OVERALL COORDINATE ERROR.
REMARK    3    ESU BASED ON R VALUE                       (A):   0.195
REMARK    3    ESU BASED ON FREE R VALUE                  (A):   0.173
REMARK    3    ESU BASED ON MAXIMUM LIKELIHOOD            (A):   0.131
REMARK    3    ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2):   5.040
REMARK    3
REMARK    3 CORRELATION COEFFICIENTS.
REMARK    3    CORRELATION COEFFICIENT FO-FC     :   0.957
REMARK    3    CORRELATION COEFFICIENT FO-FC FREE :   0.946
REMARK    3
REMARK    3 RMS DEVIATIONS FROM IDEAL VALUES        COUNT    RMS     WEIGHT
REMARK    3    BOND LENGTHS REFINED ATOMS        (A): 2506 ; 0.014 ; 0.021
REMARK    3    BOND LENGTHS OTHERS               (A): 2282 ; 0.002 ; 0.020
REMARK    3    BOND ANGLES REFINED ATOMS    (DEGREES): 3376 ; 1.501 ; 1.953
REMARK    3    BOND ANGLES OTHERS           (DEGREES): 5327 ; 0.947 ; 3.000
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99

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REMARK   3   TORSION ANGLES, PERIOD 1    (DEGREES):    297 ; 5.771 ; 5.000
REMARK   3   CHIRAL-CENTER RESTRAINTS        (A**3):    382 ; 0.086 ; 0.200
REMARK   3   GENERAL PLANES REFINED ATOMS      (A):   2718 ; 0.010 ; 0.020
REMARK   3   GENERAL PLANES OTHERS             (A):    489 ; 0.036 ; 0.020
REMARK   3   NON-BONDED CONTACTS REFINED ATOMS (A):    643 ; 0.239 ; 0.200
REMARK   3   NON-BONDED CONTACTS OTHERS        (A):   2492 ; 0.232 ; 0.200
REMARK   3   NON-BONDED TORSION OTHERS         (A):   1437 ; 0.087 ; 0.200
REMARK   3   H-BOND (X...Y) REFINED ATOMS      (A):     72 ; 0.156 ; 0.200
REMARK   3   POTENTIAL METAL-ION REFINED ATOMS (A):      1 ; 0.041 ; 0.200
REMARK   3   SYMMETRY VDW REFINED ATOMS        (A):     40 ; 0.636 ; 0.200
REMARK   3   SYMMETRY VDW OTHERS               (A):     74 ; 0.393 ; 0.200
REMARK   3   SYMMETRY H-BOND REFINED ATOMS     (A):     18 ; 0.558 ; 0.200
REMARK   3
REMARK   3   ISOTROPIC THERMAL FACTOR RESTRAINTS.    COUNT    RMS   WEIGHT
REMARK   3   MAIN-CHAIN BOND REFINED ATOMS  (A**2):   1502 ; 0.616 ; 1.500
REMARK   3   MAIN-CHAIN ANGLE REFINED ATOMS (A**2):   2417 ; 1.182 ; 2.000
REMARK   3   SIDE-CHAIN BOND REFINED ATOMS  (A**2):   1004 ; 1.967 ; 3.000
REMARK   3   SIDE-CHAIN ANGLE REFINED ATOMS (A**2):    959 ; 3.150 ; 4.500
REMARK   3
REMARK   3   NCS RESTRAINTS STATISTICS
REMARK   3   NUMBER OF DIFFERENT NCS GROUPS :    1
REMARK   3
REMARK   3   NCS GROUP NUMBER                   :    1
REMARK   3      CHAIN NAMES                     : A B
REMARK   3      NUMBER OF COMPONENTS NCS GROUP :      3
REMARK   3        COMPONENT C  SSSEQI  TO  C   SSSEQI    CODE
REMARK   3            1      A    534      A    657       6
REMARK   3            1      B    534      B    657       6
REMARK   3            2      A    672      A    686       6
REMARK   3            2      B    672      B    686       6
REMARK   3            3      A    687      A    789       6
REMARK   3            3      B    687      B    789       6
REMARK   3                  GROUP CHAIN          COUNT    RMS     WEIGHT
REMARK   3   LOOSE POSITIONAL   1     A   (A):     11 ;  0.14 ;  5.00
REMARK   3   LOOSE THERMAL      1     A (A**2):     11 ;  4.73 ; 10.00
REMARK   3
REMARK   3
REMARK   3   TLS DETAILS
REMARK   3   NUMBER OF TLS GROUPS  :    2
REMARK   3
REMARK   3   TLS GROUP :     1
REMARK   3   NUMBER OF COMPONENTS GROUP :    4
REMARK   3   COMPONENTS         C SSSEQI    TO C SSSEQI
REMARK   3   RESIDUE RANGE :    A    534      A    657
REMARK   3   RESIDUE RANGE :    A    672      A    686
REMARK   3   RESIDUE RANGE :    A    687      A    789
REMARK   3   RESIDUE RANGE :    A    804      A    862
REMARK   3   ORIGIN FOR THE GROUP (A): 29.9285   0.5264   7.4989
REMARK   3   T TENSOR
REMARK   3      T11:   0.1470 T22:   0.1360
REMARK   3      T33:   0.1011 T12:   0.0029
REMARK   3      T13:   0.0027 T23:  -0.1172
REMARK   3   L TENSOR
REMARK   3      L11:   4.8960 L22:   2.7854
REMARK   3      L33:   1.2544 L12:   0.7354
REMARK   3      L13:  -0.5427 L23:  -0.0242
REMARK   3   S TENSOR
REMARK   3      S11:   0.3148 S12:  -0.0276 S13:   0.1681
REMARK   3      S21:   0.0297 S22:  -0.3301 S23:   0.3850
REMARK   3      S31:  -0.0433 S32:  -0.0292 S33:   0.0152
REMARK   3
REMARK   3   TLS GROUP :     2
REMARK   3   NUMBER OF COMPONENTS GROUP :     1
REMARK   3   COMPONENTS         C SSSEQI    TO C SSSEQI
REMARK   3   RESIDUE RANGE :    B    686      B    686
REMARK   3   ORIGIN FOR THE GROUP (A): 28.9451 -15.8239   8.3689
REMARK   3   T TENSOR
```

```
REMARK   3      T11:   0.3135 T22:   0.3143
REMARK   3      T33:   0.3134 T12:  -0.0006
REMARK   3      T13:   0.0010 T23:   0.0001
REMARK   3    L TENSOR
REMARK   3      L11:  20.8295 L22:  24.8368
REMARK   3      L33:  60.0722 L12:  -4.3409
REMARK   3      L13: -27.2632 L23:  -5.7801
REMARK   3    S TENSOR
REMARK   3      S11:   0.0102 S12:  -0.2842 S13:   0.2176
REMARK   3      S21:   0.1711 S22:  -0.0107 S23:  -0.6694
REMARK   3      S31:  -0.3272 S32:   1.0664 S33:   0.0006
REMARK   3
REMARK   3
REMARK   3 BULK SOLVENT MODELLING.
REMARK   3  METHOD USED : BABINET MODEL WITH MASK
REMARK   3  PARAMETERS FOR MASK CALCULATION
REMARK   3  VDW PROBE RADIUS   :   1.40
REMARK   3  ION PROBE RADIUS   :   0.80
REMARK   3  SHRINKAGE RADIUS   :   0.80
REMARK   3
REMARK   3 OTHER REFINEMENT REMARKS:
REMARK   3 HYDROGENS HAVE BEEN ADDED IN THE RIDING POSITIONS
REMARK   3
LINK              HIS A 657                  LEU A 672        gap
LINK              GLN A 789                  LEU A 804        gap
CRYST1   96.411   96.411   79.026  90.00   90.00 120.00 P 62
SCALE1      0.010372  0.005988  0.000000        0.00000
SCALE2      0.000000  0.011977  0.000000        0.00000
SCALE3      0.000000  0.000000  0.012654        0.00000
ATOM      1  N   GLU A 534      13.637  -6.977  34.115  1.00 46.59           N
ATOM      3  CA  GLU A 534      14.989  -7.244  33.549  1.00 46.71           C
ATOM      5  CB  GLU A 534      15.061  -8.661  32.955  1.00 46.74           C
ATOM      8  CG  GLU A 534      16.480  -9.164  32.666  1.00 46.21           C
ATOM     11  CD  GLU A 534      16.501 -10.377  31.756  1.00 45.94           C
ATOM     12  OE1 GLU A 534      15.606 -11.233  31.869  1.00 48.25           O
ATOM     13  OE2 GLU A 534      17.409 -10.481  30.922  1.00 47.41           O
ATOM     14  C   GLU A 534      15.355  -6.213  32.478  1.00 47.04           C
ATOM     15  O   GLU A 534      14.494  -5.684  .31.767 1.00 47.18           O
ATOM     18  N   GLU A 535      16.652  -5.954  32.367  1.00 47.23           N
ATOM     20  CA  GLU A 535      17.198  -5.021  31.392  1.00 47.53           C
ATOM     22  CB  GLU A 535      18.708  -4.862  31.658  1.00 47.84           C
ATOM     25  CG  GLU A 535      19.347  -3.589  31.116  1.00 48.85           C
ATOM     28  CD  GLU A 535      20.293  -3.849  29.958  1.00 51.55           C
ATOM     29  OE1 GLU A 535      19.948  -4.658  29.058  1.00 53.04           O
ATOM     30  OE2 GLU A 535      21.386  -3.234  29.945  1.00 53.75           O
ATOM     31  C   GLU A 535      16.954  -5.432  29.918  1.00 47.41           C
ATOM     32  O   GLU A 535      16.977  -4.580  29.022  1.00 47.72           O
ATOM     33  N   GLU A 536      16.721  -6.720  29.661  1.00 47.05           N
ATOM     35  CA  GLU A 536      16.712  -7.237  28.282  1.00 46.74           C
ATOM     37  CB  GLU A 536      17.271  -8.664  28.272  1.00 47.26           C
ATOM     40  CG  GLU A 536      17.608  -9.202  26.890  1.00 49.14           C
ATOM     43  CD  GLU A 536      18.981  -9.865  26.810  1.00 52.58           C
ATOM     44  OE1 GLU A 536      19.163 -10.733  25.916  1.00 55.06           O
ATOM     45  OE2 GLU A 536      19.884  -9.510  27.615  1.00 53.60           O
ATOM     46  C   GLU A 536      15.320  -7.214  27.641  1.00 45.58           C
ATOM     47  O   GLU A 536      15.148  -6.735  26.517  1.00 45.20           O
ATOM     48  N   THR A 537      14.339  -7.747  28.363  1.00 44.47           N
ATOM     50  CA  THR A 537      12.949  -7.784  27.903  1.00 43.56           C
ATOM     52  CB  THR A 537      12.045  -8.452  28.958  1.00 43.67           C
ATOM     54  OG1 THR A 537      12.382  -7.963  30.264  1.00 44.49           O
ATOM     56  CG2 THR A 537      12.289  -9.955  29.035  1.00 44.07           C
ATOM     60  C   THR A 537      12.388  -6.394  27.619  1.00 42.43           C
ATOM     61  O   THR A 537      11.610  -6.221  26.689  1.00 42.65           O
ATOM     62  N   ARG A 538      12.769  -5.413  28.433  1.00 41.09           N
ATOM     64  CA  ARG A 538      12.226  -4.062  28.303  1.00 40.04           C
ATOM     66  CB  ARG A 538      12.529  -3.221  29.552  1.00 40.12           C
ATOM     69  CG  ARG A 538      11.534  -3.401  30.690  1.00 39.78           C
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ATOM     72  CD   ARG A 538      11.540  -2.271  31.725  1.00 40.15           C
ATOM     75  NE   ARG A 538      11.185  -2.748  33.070  1.00 40.21           N
ATOM     77  CZ   ARG A 538      12.031  -3.339  33.918  1.00 40.09           C
ATOM     78  NH1  ARG A 538      13.302  -3.546  33.583  1.00 40.79           N
ATOM     81  NH2  ARG A 538      11.605  -3.728  35.111  1.00 40.74           N
ATOM     84  C    ARG A 538      12.721  -3.345  27.038  1.00 39.10           C
ATOM     85  O    ARG A 538      11.927  -2.719  26.339  1.00 38.29           O
ATOM     86  N    GLU A 539      14.013  -3.439  26.729  1.00 38.12           N
ATOM     88  CA   GLU A 539      14.513  -2.825  25.510  1.00 37.97           C
ATOM     90  CB   GLU A 539      15.985  -3.171  25.262  1.00 38.18           C
ATOM     93  CG   GLU A 539      16.978  -2.098  25.671  1.00 39.15           C
ATOM     96  CD   GLU A 539      18.404  -2.487  25.300  1.00 40.30           C
ATOM     97  OE1  GLU A 539      18.552  -3.462  24.550  1.00 40.77           O
ATOM     98  OE2  GLU A 539      19.367  -1.846  25.778  1.00 42.40           O
ATOM     99  C    GLU A 539      13.688  -3.284  24.309  1.00 37.73           C
ATOM    100  O    GLU A 539      13.366  -2.489  23.425  1.00 37.24           O
ATOM    101  N    LEU A 540      13.348  -4.573  24.284  1.00 37.69           N
ATOM    103  CA   LEU A 540      12.592  -5.156  23.177  1.00 37.31           C
ATOM    105  CB   LEU A 540      12.498  -6.677  23.339  1.00 37.36           C
ATOM    108  CG   LEU A 540      12.356  -7.576  22.110  1.00 37.64           C
ATOM    110  CD1  LEU A 540      11.465  -8.736  22.453  1.00 37.32           C
ATOM    114  CD2  LEU A 540      11.849  -6.885  20.847  1.00 38.06           C
ATOM    118  C    LEU A 540      11.196  -4.548  23.060  1.00 37.13           C
ATOM    119  O    LEU A 540      10.781  -4.169  21.975  1.00 36.13           O
ATOM    120  N    GLN A 541      10.475  -4.468  24.178  1.00 38.03           N
ATOM    122  CA   GLN A 541       9.124  -3.896  24.200  1.00 38.21           C
ATOM    124  CB   GLN A 541       8.564  -3.886  25.624  1.00 38.94           C
ATOM    127  CG   GLN A 541       8.343  -5.265  26.267  1.00 40.24           C
ATOM    130  CD   GLN A 541       7.319  -6.120  25.543  1.00 41.38           C
ATOM    131  OE1  GLN A 541       6.254  -6.416  26.097  1.00 43.49           O
ATOM    132  NE2  GLN A 541       7.650  -6.550  24.318  1.00 42.15           N
ATOM    135  C    GLN A 541       9.106  -2.471  23.643  1.00 38.18           C
ATOM    136  O    GLN A 541       8.320  -2.163  22.751  1.00 38.20           O
ATOM    137  N    SER A 542       9.977  -1.616  24.176  1.00 38.16           N
ATOM    139  CA   SER A 542      10.171  -0.259  23.658  1.00 38.24           C
ATOM    141  CB   SER A 542      11.413   0.405  24.294  1.00 38.29           C
ATOM    144  OG   SER A 542      11.168   0.706  25.667  1.00 40.50           O
ATOM    146  C    SER A 542      10.346  -0.268  22.146  1.00 37.34           C
ATOM    147  O    SER A 542       9.620   0.397  21.419  1.00 37.32           O
ATOM    148  N    LEU A 543      11.303  -1.050  21.676  1.00 36.77           N
ATOM    150  CA   LEU A 543      11.681  -1.020  20.268  1.00 36.27           C
ATOM    152  CB   LEU A 543      12.926  -1.889  20.028  1.00 35.78           C
ATOM    155  CG   LEU A 543      13.961  -1.395  19.028  1.00 35.13           C
ATOM    157  CD1  LEU A 543      14.656  -2.564  18.401  1.00 35.18           C
ATOM    161  CD2  LEU A 543      13.429  -0.468  17.943  1.00 35.56           C
ATOM    165  C    LEU A 543      10.552  -1.488  19.353  1.00 36.22           C
ATOM    166  O    LEU A 543      10.233  -0.838  18.364  1.00 36.70           O
ATOM    167  N    ALA A 544       9.980  -2.636  19.681  1.00 36.30           N
ATOM    169  CA   ALA A 544       8.928  -3.259  18.885  1.00 36.40           C
ATOM    171  CB   ALA A 544       8.591  -4.635  19.455  1.00 36.48           C
ATOM    175  C    ALA A 544       7.663  -2.416  18.793  1.00 36.41           C
ATOM    176  O    ALA A 544       6.968  -2.451  17.779  1.00 36.62           O
ATOM    177  N    ALA A 545       7.363  -1.662  19.842  1.00 36.50           N
ATOM    179  CA   ALA A 545       6.118  -0.890  19.887  1.00 36.47           C
ATOM    181  CB   ALA A 545       5.498  -0.939  21.290  1.00 36.49           C
ATOM    185  C    ALA A 545       6.352   0.554  19.459  1.00 36.41           C
ATOM    186  O    ALA A 545       5.414   1.316  19.365  1.00 35.99           O
ATOM    187  N    ALA A 546       7.599   0.929  19.164  1.00 36.49           N
ATOM    189  CA   ALA A 546       7.867   2.315  18.792  1.00 36.48           C
ATOM    191  CB   ALA A 546       9.334   2.687  19.042  1.00 36.01           C
ATOM    195  C    ALA A 546       7.480   2.532  17.338  1.00 36.52           C
ATOM    196  O    ALA A 546       7.583   1.618  16.524  1.00 36.69           O
ATOM    197  N    VAL A 547       6.991   3.731  17.037  1.00 36.54           N
ATOM    199  CA   VAL A 547       6.740   4.160  15.667  1.00 36.75           C
ATOM    201  CB   VAL A 547       5.976   5.520  15.623  1.00 37.32           C
ATOM    203  CG1  VAL A 547       5.990   6.140  14.214  1.00 38.11           C
ATOM    207  CG2  VAL A 547       4.547   5.353  16.124  1.00 37.43           C
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ATOM	211	C	VAL A 547	8.089	4.313	14.996	1.00 36.37	C
ATOM	212	O	VAL A 547	8.997	4.931	15.547	1.00 37.11	O
ATOM	213	N	VAL A 548	8.247	3.700	13.830	1.00 35.84	N
ATOM	215	CA	VAL A 548	9.474	3.842	13.059	1.00 34.92	C
ATOM	217	CB	VAL A 548	9.743	2.597	12.203	1.00 35.05	C
ATOM	219	CG1	VAL A 548	11.064	2.729	11.489	1.00 34.26	C
ATOM	223	CG2	VAL A 548	9.721	1.338	13.065	1.00 35.00	C
ATOM	227	C	VAL A 548	9.303	5.069	12.167	1.00 34.49	C
ATOM	228	O	VAL A 548	8.531	5.019	11.219	1.00 34.01	O
ATOM	229	N	PRO A 549	9.976	6.180	12.459	1.00 33.90	N
ATOM	230	CA	PRO A 549	9.824	7.360	11.603	1.00 33.96	C
ATOM	232	CB	PRO A 549	10.636	8.456	12.325	1.00 33.76	C
ATOM	235	CG	PRO A 549	10.862	7.955	13.695	1.00 34.32	C
ATOM	238	CD	PRO A 549	10.901	6.438	13.574	1.00 34.16	C
ATOM	241	C	PRO A 549	10.351	7.104	10.195	1.00 33.88	C
ATOM	242	O	PRO A 549	11.107	6.139	9.961	1.00 34.16	O
ATOM	243	N	SER A 550	9.952	7.968	9.267	1.00 33.64	N
ATOM	245	CA	SER A 550	10.307	7.816	7.871	1.00 34.15	C
ATOM	247	CB	SER A 550	9.515	8.800	7.022	1.00 34.02	C
ATOM	250	OG	SER A 550	10.072	10.094	7.126	1.00 33.86	O
ATOM	252	C	SER A 550	11.807	8.026	7.653	1.00 34.44	C
ATOM	253	O	SER A 550	12.498	8.601	8.480	1.00 35.07	O
ATOM	254	N	ALA A 551	12.309	7.537	6.536	1.00 34.56	N
ATOM	256	CA	ALA A 551	13.690	7.773	6.158	1.00 34.63	C
ATOM	258	CB	ALA A 551	13.983	7.122	4.805	1.00 34.69	C
ATOM	262	C	ALA A 551	14.025	9.262	6.117	1.00 34.98	C
ATOM	263	O	ALA A 551	15.087	9.675	6.604	1.00 34.64	O
ATOM	264	N	GLN A 552	13.134	10.049	5.518	1.00 35.27	N
ATOM	266	CA	GLN A 552	13.305	11.495	5.424	1.00 36.31	C
ATOM	268	CB	GLN A 552	12.108	12.129	4.698	1.00 36.61	C
ATOM	271	CG	GLN A 552	12.240	13.658	4.434	1.00 38.76	C
ATOM	274	CD	GLN A 552	10.971	14.236	3.807	1.00 41.89	C
ATOM	275	OE1	GLN A 552	10.537	13.767	2.751	1.00 43.18	O
ATOM	276	NE2	GLN A 552	10.361	15.230	4.465	1.00 44.04	N
ATOM	279	C	GLN A 552	13.476	12.162	6.800	1.00 36.32	C
ATOM	280	O	GLN A 552	14.398	12.938	6.990	1.00 36.63	O
ATOM	281	N	THR A 553	12.566	11.868	7.725	1.00 36.31	N
ATOM	283	CA	THR A 553	12.619	12.377	9.098	1.00 36.39	C
ATOM	285	CB	THR A 553	11.422	11.841	9.922	1.00 36.22	C
ATOM	287	OG1	THR A 553	10.178	12.197	9.301	1.00 36.41	O
ATOM	289	CG2	THR A 553	11.359	12.516	11.300	1.00 36.57	C
ATOM	293	C	THR A 553	13.916	11.958	9.804	1.00 36.49	C
ATOM	294	O	THR A 553	14.472	12.719	10.589	1.00 36.70	O
ATOM	295	N	LEU A 554	14.390	10.747	9.541	1.00 36.10	N
ATOM	297	CA	LEU A 554	15.578	10.252	10.242	1.00 36.39	C
ATOM	299	CB	LEU A 554	15.532	8.735	10.373	1.00 36.37	C
ATOM	302	CG	LEU A 554	14.432	8.145	11.243	1.00 36.80	C
ATOM	304	CD1	LEU A 554	14.471	6.628	11.089	1.00 37.50	C
ATOM	308	CD2	LEU A 554	14.582	8.566	12.701	1.00 36.05	C
ATOM	312	C	LEU A 554	16.905	10.692	9.604	1.00 36.32	C
ATOM	313	O	LEU A 554	17.956	10.487	10.191	1.00 36.86	O
ATOM	314	N	LYS A 555	16.845	11.311	8.427	1.00 36.57	N
ATOM	316	CA	LYS A 555	18.021	11.840	7.727	1.00 36.89	C
ATOM	318	CB	LYS A 555	18.772	12.862	8.597	1.00 37.14	C
ATOM	321	CG	LYS A 555	17.922	13.972	9.133	1.00 38.89	C
ATOM	324	CD	LYS A 555	18.742	14.950	9.972	1.00 42.02	C
ATOM	327	CE	LYS A 555	17.839	16.017	10.608	1.00 43.95	C
ATOM	330	NZ	LYS A 555	18.583	17.280	11.057	1.00 46.49	N
ATOM	334	C	LYS A 555	19.007	10.769	7.275	1.00 36.71	C
ATOM	335	O	LYS A 555	20.136	11.089	6.959	1.00 37.24	O
ATOM	336	N	ILE A 556	18.592	9.507	7.242	1.00 36.27	N
ATOM	338	CA	ILE A 556	19.500	8.415	6.944	1.00 35.92	C
ATOM	340	CB	ILE A 556	18.930	7.099	7.436	1.00 36.15	C
ATOM	342	CG1	ILE A 556	17.578	6.808	6.762	1.00 35.82	C
ATOM	345	CD1	ILE A 556	17.211	5.371	6.785	1.00 36.65	C
ATOM	349	CG2	ILE A 556	18.808	7.132	8.926	1.00 37.25	C
ATOM	353	C	ILE A 556	19.875	8.267	5.476	1.00 35.83	C

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ATOM    354  O    ILE A 556     20.796    7.523    5.164  1.00 35.57          O
ATOM    355  N    THR A 557     19.145    8.925    4.585  1.00 36.11          N
ATOM    357  CA   THR A 557     19.498    8.982    3.163  1.00 37.13          C
ATOM    359  CB   THR A 557     18.291    9.458    2.315  1.00 37.47          C
ATOM    361  OG1  THR A 557     17.101    8.764    2.713  1.00 38.12          O
ATOM    363  CG2  THR A 557     18.447    9.061    0.838  1.00 38.48          C
ATOM    367  C    THR A 557     20.707    9.891    2.904  1.00 37.31          C
ATOM    368  O    THR A 557     21.322    9.813    1.846  1.00 37.89          O
ATOM    369  N    ASP A 558     21.067   10.717    3.885  1.00 37.34          N
ATOM    371  CA   ASP A 558     22.131   11.705    3.731  1.00 37.90          C
ATOM    373  CB   ASP A 558     21.983   12.865    4.748  1.00 38.69          C
ATOM    376  CG   ASP A 558     20.629   13.574    4.660  1.00 41.60          C
ATOM    377  OD1  ASP A 558     19.745   13.072    3.924  1.00 46.07          O
ATOM    378  OD2  ASP A 558     20.363   14.638    5.284  1.00 44.02          O
ATOM    379  C    ASP A 558     23.501   11.103    3.942  1.00 37.29          C
ATOM    380  O    ASP A 558     23.745   10.454    4.960  1.00 36.55          O
ATOM    381  N    PHE A 559     24.409   11.371    3.009  1.00 36.83          N
ATOM    383  CA   PHE A 559     25.811   11.066    3.216  1.00 37.19          C
ATOM    385  CB   PHE A 559     26.616   11.405    1.961  1.00 37.33          C
ATOM    388  CG   PHE A 559     26.453   10.399    0.852  1.00 36.15          C
ATOM    389  CD1  PHE A 559     25.982   10.791   -0.392  1.00 34.77          C
ATOM    391  CE1  PHE A 559     25.840    9.878   -1.408  1.00 34.14          C
ATOM    393  CZ   PHE A 559     26.139    8.550   -1.191  1.00 32.43          C
ATOM    395  CE2  PHE A 559     26.609    8.131    0.050  1.00 33.75          C
ATOM    397  CD2  PHE A 559     26.759    9.062   ·1.066  1.00 35.34          C
ATOM    399  C    PHE A 559     26.397   11.784    4.458  1.00 37.80          C
ATOM    400  O    PHE A 559     27.284   11.258    5.139  1.00 38.12          O
ATOM    401  N    SER A 560     25.858   12.947    4.757  1.00 37.70          N
ATOM    403  CA   SER A 560     26.293   13.784    5.879  1.00 39.03          C
ATOM    405  CB   SER A 560     25.835   15.215    5.579  1.00 39.04          C
ATOM    408  OG   SER A 560     26.651   15.717    4.538  1.00 41.01          O
ATOM    410  C    SER A 560     25.807   13.405    7.300  1.00 39.09          C
ATOM    411  O    SER A 560     26.199   14.033    8.285  1.00 39.31          O
ATOM    412  N    PHE A 561     24.947   12.402    7.382  1.00 39.13          N
ATOM    414  CA   PHE A 561     24.382   11.907    8.629  1.00 38.97          C
ATOM    416  CB   PHE A 561     23.823   10.514    8.364  1.00 38.71          C
ATOM    419  CG   PHE A 561     23.201    9.868    9.550  1.00 37.66          C
ATOM    420  CD1  PHE A 561     21.877   10.112    9.864  1.00 36.57          C
ATOM    422  CE1  PHE A 561     21.280    9.486   10.925  1.00 36.89          C
ATOM    424  CZ   PHE A 561     22.004    8.579   11.680  1.00 37.15          C
ATOM    426  CE2  PHE A 561     23.319    8.309   11.380  1.00 35.98          C
ATOM    428  CD2  PHE A 561     23.922    8.947   10.310  1.00 37.17          C
ATOM    430  C    PHE A 561     25.392   11.802    9.755  1.00 39.21          C
ATOM    431  O    PHE A 561     26.518   11.347    9.516  1.00 38.44          O
ATOM    432  N    SER A 562     24.970   12.166   10.975  1.00 39.53          N
ATOM    434  CA   SER A 562     25.845   12.056   12.148  1.00 40.79          C
ATOM    436  CB   SER A 562     25.934   13.376   12.916  1.00 41.03          C
ATOM    439  OG   SER A 562     26.984   13.244   13.855  1.00 40.22          O
ATOM    441  C    SER A 562     25.615   10.895   13.153  1.00 41.03          C
ATOM    442  O    SER A 562     26.440   10.016   13.224  1.00 45.01          O
ATOM    443  N    ASP A 563     24.573   10.864   13.933  1.00 40.48          N
ATOM    445  CA   ASP A 563     24.485    9.895   15.082  1.00 40.78          C
ATOM    447  CB   ASP A 563     24.996    8.476   14.761  1.00 40.79          C
ATOM    450  CG   ASP A 563     26.311    8.095   15.496  1.00 41.91          C
ATOM    451  OD1  ASP A 563     27.385    8.617   15.150  1.00 42.85          O
ATOM    452  OD2  ASP A 563     26.383    7.226   16.396  1.00 42.36          O
ATOM    453  C    ASP A 563     25.019   10.357   16.478  1.00 40.06          C
ATOM    454  O    ASP A 563     24.619    9.825   17.504  1.00 38.82          O
ATOM    455  N    PHE A 564     25.904   11.340   16.513  1.00 39.33          N
ATOM    457  CA   PHE A 564     26.386   11.857   17.795  1.00 39.36          C
ATOM    459  CB   PHE A 564     27.203   13.141   17.603  1.00 39.66          C
ATOM    462  CG   PHE A 564     28.657   12.870   17.381  1.00 43.24          C
ATOM    463  CD1  PHE A 564     29.200   12.874   16.098  1.00 47.67          C
ATOM    465  CE1  PHE A 564     30.563   12.592   15.875  1.00 47.93          C
ATOM    467  CZ   PHE A 564     31.378   12.287   16.947  1.00 48.79          C
ATOM    469  CE2  PHE A 564     30.834   12.254   18.254  1.00 49.45          C
ATOM    471  CD2  PHE A 564     29.478   12.544   18.455  1.00 47.83          C
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ATOM    473  C    PHE A 564    25.261  12.052  18.811  1.00 37.82    C
ATOM    474  O    PHE A 564    25.335  11.532  19.915  1.00 38.99    O
ATOM    475  N    GLU A 565    24.192  12.714  18.402  1.00 35.36    N
ATOM    477  CA   GLU A 565    23.147  13.112  19.326  1.00 33.95    C
ATOM    479  CB   GLU A 565    22.502  14.398  18.801  1.00 34.14    C
ATOM    482  CG   GLU A 565    23.501  15.529  18.592  1.00 34.68    C
ATOM    485  CD   GLU A 565    24.101  16.014  19.908  1.00 36.90    C
ATOM    486  OE1  GLU A 565    23.466  16.857  20.627  1.00 35.83    O
ATOM    487  OE2  GLU A 565    25.209  15.534  20.224  1.00 37.58    O
ATOM    488  C    GLU A 565    22.088  12.037  19.601  1.00 31.86    C
ATOM    489  O    GLU A 565    21.295  12.176  20.524  1.00 29.83    O
ATOM    490  N    LEU A 566    22.100  10.964  18.819  1.00 30.24    N
ATOM    492  CA   LEU A 566    21.068   9.928  18.904  1.00 29.66    C
ATOM    494  CB   LEU A 566    20.992   9.104  17.604  1.00 29.51    C
ATOM    497  CG   LEU A 566    20.779   9.878  16.294  1.00 30.52    C
ATOM    499  CD1  LEU A 566    20.689   8.857  15.168  1.00 32.77    C
ATOM    503  CD2  LEU A 566    19.525  10.745  16.304  1.00 32.40    C
ATOM    507  C    LEU A 566    21.274   8.966  20.059  1.00 28.90    C
ATOM    508  O    LEU A 566    22.398   8.657  20.420  1.00 29.20    O
ATOM    509  N    SER A 567    20.171   8.507  20.629  1.00 27.75    N
ATOM    511  CA   SER A 567    20.153   7.424  21.601  1.00 27.51    C
ATOM    513  CB   SER A 567    18.820   7.439  22.353  1.00 27.43    C
ATOM    516  OG   SER A 567    17.737   7.060  21.502  1.00 25.32    O
ATOM    518  C    SER A 567    20.298   6.072  20.908  1.00 28.05    C
ATOM    519  O    SER A 567    20.132   5.978  19.682  1.00 28.56    O
ATOM    520  N    ASP A 568    20.573   5.021  21.674  1.00 28.29    N
ATOM    522  CA   ASP A 568    20.710   3.684  21.078  1.00 29.26    C
ATOM    524  CB   ASP A 568    21.188   2.640  22.108  1.00 29.83    C
ATOM    527  CG   ASP A 568    22.661   2.767  22.434  1.00 29.32    C
ATOM    528  OD1  ASP A 568    23.368   3.481  21.729  1.00 29.71    O
ATOM    529  OD2  ASP A 568    23.187   2.210  23.405  1.00 32.67    O
ATOM    530  C    ASP A 568    19.376   3.227  20.468  1.00 29.47    C
ATOM    531  O    ASP A 568    19.348   2.608  19.410  1.00 27.71    O
ATOM    532  N    LEU A 569    18.283   3.536  21.155  1.00 29.32    N
ATOM    534  CA   LEU A 569    16.958   3.252  20.624  1.00 29.88    C
ATOM    536  CB   LEU A 569    15.869   3.644  21.619  1.00 29.64    C
ATOM    539  CG   LEU A 569    14.454   3.796  21.041  1.00 31.25    C
ATOM    541  CD1  LEU A 569    13.901   2.447  20.638  1.00 32.64    C
ATOM    545  CD2  LEU A 569    13.527   4.504  22.040  1.00 33.35    C
ATOM    549  C    LEU A 569    16.738   3.950  19.273  1.00 29.85    C
ATOM    550  O    LEU A 569    16.241   3.314  18.340  1.00 30.01    O
ATOM    551  N    GLU A 570    17.098   5.235  19.167  1.00 29.92    N
ATOM    553  CA   GLU A 570    16.980   5.962  17.894  1.00 30.00    C
ATOM    555  CB   GLU A 570    17.325   7.451  18.031  1.00 30.17    C
ATOM    558  CG   GLU A 570    16.156   8.339  18.478  1.00 30.37    C
ATOM    561  CD   GLU A 570    16.589   9.717  18.960  1.00 32.39    C
ATOM    562  OE1  GLU A 570    17.695   9.844  19.501  1.00 30.13    O
ATOM    563  OE2  GLU A 570    15.814  10.692  18.831  1.00 37.62    O
ATOM    564  C    GLU A 570    17.798   5.296  16.770  1.00 30.28    C
ATOM    565  O    GLU A 570    17.308   5.200  15.629  1.00 30.35    O
ATOM    566  N    THR A 571    19.001   4.794  17.080  1.00 29.57    N
ATOM    568  CA   THR A 571    19.794   4.091  16.066  1.00 29.67    C
ATOM    570  CB   THR A 571    21.272   3.816  16.506  1.00 29.48    C
ATOM    572  OG1  THR A 571    21.314   2.935  17.629  1.00 28.30    O
ATOM    574  CG2  THR A 571    21.957   5.100  16.974  1.00 28.02    C
ATOM    578  C    THR A 571    19.130   2.784  15.640  1.00 29.33    C
ATOM    579  O    THR A 571    19.221   2.379  14.466  1.00 30.05    O
ATOM    580  N    ALA A 572    18.492   2.124  16.590  1.00 29.18    N
ATOM    582  CA   ALA A 572    17.756   0.891  16.318  1.00 29.80    C
ATOM    584  CB   ALA A 572    17.308   0.240  17.602  1.00 29.64    C
ATOM    588  C    ALA A 572    16.567   1.167  15.401  1.00 30.05    C
ATOM    589  O    ALA A 572    16.316   0.391  14.495  1.00 30.93    O
ATOM    590  N    LEU A 573    15.869   2.277  15.612  1.00 30.31    N
ATOM    592  CA   LEU A 573    14.760   2.688  14.741  1.00 31.26    C
ATOM    594  CB   LEU A 573    13.975   3.869  15.344  1.00 31.12    C
ATOM    597  CG   LEU A 573    13.214   3.543  16.635  1.00 32.60    C
ATOM    599  CD1  LEU A 573    12.544   4.815  17.262  1.00 33.72    C
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ATOM    603  CD2 LEU A 573      12.173    2.452   16.404  1.00 31.36           C
ATOM    607  C   LEU A 573      15.291    3.033   13.339  1.00 31.87           C
ATOM    608  O   LEU A 573      14.715    2.619   12.325  1.00 31.11           O
ATOM    609  N   CYS A 574      16.420    3.735   13.286  1.00 31.88           N
ATOM    611  CA  CYS A 574      17.080    3.994   12.016  1.00 32.11           C
ATOM    613  CB  CYS A 574      18.368    4.798   12.228  1.00 32.66           C
ATOM    616  SG  CYS A 574      18.138    6.536   12.617  1.00 33.97           S
ATOM    617  C   CYS A 574      17.390    2.690   11.277  1.00 32.33           C
ATOM    618  O   CYS A 574      17.255    2.609   10.047  1.00 31.91           O
ATOM    619  N   THR A 575      17.804    1.669   12.023  1.00 32.03           N
ATOM    621  CA  THR A 575      18.196    0.407   11.427  1.00 31.37           C
ATOM    623  CB  THR A 575      18.929   -0.460   12.452  1.00 31.74           C
ATOM    625  OG1 THR A 575      20.116    0.231   12.896  1.00 30.30           O
ATOM    627  CG2 THR A 575      19.419   -1.765   11.804  1.00 32.94           C
ATOM    631  C   THR A 575      16.979   -0.332   10.864  1.00 31.23           C
ATOM    632  O   THR A 575      17.053   -0.878    9.783  1.00 30.31           O
ATOM    633  N   ILE A 576      15.866   -0.317   11.589  1.00 30.87           N
ATOM    635  CA  ILE A 576      14.598   -0.848   11.083  1.00 31.22           C
ATOM    637  CB  ILE A 576      13.497   -0.787   12.139  1.00 30.84           C
ATOM    639  CG1 ILE A 576      13.882   -1.665   13.329  1.00 30.99           C
ATOM    642  CD1 ILE A 576      12.992   -1.437   14.558  1.00 33.62           C
ATOM    646  CG2 ILE A 576      12.119   -1.233   11.549  1.00 31.41           C
ATOM    650  C   ILE A 576      14.168   -0.119    9.813  1.00 31.43           C
ATOM    651  O   ILE A 576      13.757   -0.759    8.853  1.00 31.90           O
ATOM    652  N   ARG A 577      14.301    1.194    9.780  1.00 30.79           N
ATOM    654  CA  ARG A 577      13.964    1.937    8.585  1.00 31.23           C
ATOM    656  CB  ARG A 577      14.058    3.437    8.821  1.00 31.07           C
ATOM    659  CG  ARG A 577      13.601    4.319    7.656  1.00 30.28           C
ATOM    662  CD  ARG A 577      12.315    3.885    6.972  1.00 29.17           C
ATOM    665  NE  ARG A 577      11.145    4.167    7.796  1.00 29.79           N
ATOM    667  CZ  ARG A 577       9.928    3.698    7.577  1.00 28.87           C
ATOM    668  NH1 ARG A 577       9.679    2.875    6.571  1.00 29.13           N
ATOM    671  NH2 ARG A 577       8.947    4.051    8.388  1.00 28.52           N
ATOM    674  C   ARG A 577      14.827    1.544    7.378  1.00 32.02           C
ATOM    675  O   ARG A 577      14.318    1.486    6.255  1.00 31.52           O
ATOM    676  N   MET A 578      16.117    1.299    7.602  1.00 32.31           N
ATOM    678  CA  MET A 578      17.036    0.882    6.525  1.00 32.58           C
ATOM    680  CB  MET A 578      18.449    0.716    7.061  1.00 32.59           C
ATOM    683  CG  MET A 578      19.157    2.010    7.334  1.00 35.68           C
ATOM    686  SD  MET A 578      20.767    1.762    8.093  1.00 38.66           S
ATOM    687  CE  MET A 578      20.727    3.087    9.249  1.00 37.18           C
ATOM    691  C   MET A 578      16.587   -0.447    5.895  1.00 32.70           C
ATOM    692  O   MET A 578      16.530   -0.574    4.660  1.00 33.48           O
ATOM    693  N   PHE A 579      16.270   -1.425    6.739  1.00 32.11           N
ATOM    695  CA  PHE A 579      15.767   -2.719    6.277  1.00 32.29           C
ATOM    697  CB  PHE A 579      15.582   -3.708    7.452  1.00 31.89           C
ATOM    700  CG  PHE A 579      16.839   -4.434    7.859  1.00 31.60           C
ATOM    701  CD1 PHE A 579      17.675   -3.926    8.835  1.00 33.58           C
ATOM    703  CE1 PHE A 579      18.841   -4.613    9.215  1.00 32.33           C
ATOM    705  CZ  PHE A 579      19.162   -5.807    8.612  1.00 31.30           C
ATOM    707  CE2 PHE A 579      18.321   -6.319    7.656  1.00 31.22           C
ATOM    709  CD2 PHE A 579      17.178   -5.632    7.281  1.00 31.99           C
ATOM    711  C   PHE A 579      14.441   -2.553    5.510  1.00 32.24           C
ATOM    712  O   PHE A 579      14.185   -3.257    4.523  1.00 32.02           O
ATOM    713  N   THR A 580      13.604   -1.635    5.970  1.00 32.08           N
ATOM    715  CA  THR A 580      12.278   -1.430    5.416  1.00 32.46           C
ATOM    717  CB  THR A 580      11.408   -0.621    6.410  1.00 32.61           C
ATOM    719  OG1 THR A 580      11.337   -1.312    7.668  1.00 31.75           O
ATOM    721  CG2 THR A 580       9.934   -0.550    5.950  1.00 33.38           C
ATOM    725  C   THR A 580      12.318   -0.753    4.043  1.00 32.81           C
ATOM    726  O   THR A 580      11.677   -1.215    3.108  1.00 33.63           O
ATOM    727  N   ASP A 581      13.080    0.325    3.922  1.00 33.22           N
ATOM    729  CA  ASP A 581      13.091    1.144    2.710  1.00 33.25           C
ATOM    731  CB  ASP A 581      13.577    2.557    3.023  1.00 32.63           C
ATOM    734  CG  ASP A 581      12.445    3.489    3.515  1.00 34.46           C
ATOM    735  OD1 ASP A 581      11.400    2.992    4.031  1.00 32.82           O
ATOM    736  OD2 ASP A 581      12.532    4.741    3.404  1.00 32.84           O
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ATOM    737  C   ASP A 581     13.963   0.518   1.615  1.00 33.04           C
ATOM    738  O   ASP A 581     13.937   0.956   0.482  1.00 32.09           O
ATOM    739  N   LEU A 582     14.767  -0.477   1.981  1.00 34.07           N
ATOM    741  CA  LEU A 582     15.502  -1.291   1.016  1.00 34.19           C
ATOM    743  CB  LEU A 582     16.827  -1.781   1.606  1.00 34.53           C
ATOM    746  CG  LEU A 582     17.906  -0.693   1.643  1.00 34.93           C
ATOM    748  CD1 LEU A 582     19.138  -1.108   2.422  1.00 33.63           C
ATOM    752  CD2 LEU A 582     18.282  -0.297   0.212  1.00 36.11           C
ATOM    756  C   LEU A 582     14.639  -2.472   0.590  1.00 34.48           C
ATOM    757  O   LEU A 582     15.118  -3.352  -0.109  1.00 34.23           O
ATOM    758  N   ASN A 583     13.381  -2.469   1.031  1.00 34.36           N
ATOM    760  CA  ASN A 583     12.406  -3.546   0.818  1.00 34.99           C
ATOM    762  CB  ASN A 583     12.008  -3.640  -0.668  1.00 35.09           C
ATOM    765  CG  ASN A 583     11.132  -2.495  -1.088  1.00 37.78           C
ATOM    766  OD1 ASN A 583     10.004  -2.356  -0.609  1.00 39.93           O
ATOM    767  ND2 ASN A 583     11.647  -1.645  -1.962  1.00 41.11           N
ATOM    770  C   ASN A 583     12.804  -4.914   1.360  1.00 34.32           C
ATOM    771  O   ASN A 583     12.303  -5.946   0.882  1.00 34.23           O
ATOM    772  N   LEU A 584     13.710  -4.947   2.331  1.00 33.18           N
ATOM    774  CA  LEU A 584     14.185  -6.230   2.854  1.00 33.64           C
ATOM    776  CB  LEU A 584     15.523  -6.099   3.584  1.00 33.24           C
ATOM    779  CG  LEU A 584     16.669  -5.565   2.722  1.00 32.96           C
ATOM    781  CD1 LEU A 584     17.913  -5.315   3.557  1.00 31.66           C
ATOM    785  CD2 LEU A 584     16.954  -6.513   1.562  1.00 32.83           C
ATOM    789  C   LEU A 584     13.141  -6.937   3.728  1.00 34.16           C
ATOM    790  O   LEU A 584     12.989  -8.153   3.647  1.00 34.32           O
ATOM    791  N   VAL A 585     12.410  -6.178   4.527  1.00 34.42           N
ATOM    793  CA  VAL A 585     11.444  -6.748   5.461  1.00 35.24           C
ATOM    795  CB  VAL A 585     10.860  -5.665   6.387  1.00 35.15           C
ATOM    797  CG1 VAL A 585      9.643  -6.188   7.169  1.00 35.52           C
ATOM    801  CG2 VAL A 585     11.961  -5.140   7.346  1.00 35.21           C
ATOM    805  C   VAL A 585     10.290  -7.447   4.730  1.00 36.18           C
ATOM    806  O   VAL A 585      9.806  -8.526   5.149  1.00 35.50           O
ATOM    807  N   GLN A 586      9.846  -6.823   3.647  1.00 36.68           N
ATOM    809  CA  GLN A 586      8.700  -7.340   2.930  1.00 37.07           C
ATOM    811  CB  GLN A 586      7.877  -6.192   2.338  1.00 37.74           C
ATOM    814  CG  GLN A 586      8.403  -5.499   1.083  1.00 39.21           C
ATOM    817  CD  GLN A 586      7.248  -4.869   0.298  1.00 42.46           C
ATOM    818  OE1 GLN A 586      6.256  -5.540   0.002  1.00 41.35           O
ATOM    819  NE2 GLN A 586      7.362  -3.575  -0.006  1.00 46.17           N
ATOM    822  C   GLN A 586      9.099  -8.413   1.901  1.00 36.36           C
ATOM    823  O   GLN A 586      8.424  -9.427   1.791  1.00 37.00           O
ATOM    824  N   ASN A 587     10.194  -8.206   1.176  1.00 35.61           N
ATOM    826  CA  ASN A 587     10.638  -9.191   0.176  1.00 35.71           C
ATOM    828  CB  ASN A 587     11.765  -8.615  -0.702  1.00 35.65           C
ATOM    831  CG  ASN A 587     11.305  -7.460  -1.590  1.00 36.24           C
ATOM    832  OD1 ASN A 587     10.134  -7.102  -1.611  1.00 39.75           O
ATOM    833  ND2 ASN A 587     12.247  -6.871  -2.328  1.00 36.37           N
ATOM    836  C   ASN A 587     11.114 -10.533   0.786  1.00 35.57           C
ATOM    837  O   ASN A 587     11.109 -11.579   0.104  1.00 34.41           O
ATOM    838  N   PHE A 588     11.578 -10.483   2.038  1.00 35.12           N
ATOM    840  CA  PHE A 588     12.088 -11.661   2.750  1.00 35.20           C
ATOM    842  CB  PHE A 588     13.535 -11.407   3.179  1.00 34.99           C
ATOM    845  CG  PHE A 588     14.465 -11.258   2.018  1.00 34.40           C
ATOM    846  CD1 PHE A 588     15.075 -10.060   1.740  1.00 32.27           C
ATOM    848  CE1 PHE A 588     15.895  -9.933   0.634  1.00 34.30           C
ATOM    850  CZ  PHE A 588     16.102 -11.016  -0.210  1.00 34.81           C
ATOM    852  CE2 PHE A 588     15.473 -12.207   0.045  1.00 34.03           C
ATOM    854  CD2 PHE A 588     14.652 -12.321   1.145  1.00 34.98           C
ATOM    856  C   PHE A 588     11.225 -12.084   3.948  1.00 35.69           C
ATOM    857  O   PHE A 588     11.635 -12.940   4.735  1.00 35.71           O
ATOM    858  N   GLN A 589     10.045 -11.476   4.079  1.00 36.23           N
ATOM    860  CA  GLN A 589      9.063 -11.824   5.119  1.00 37.00           C
ATOM    862  CB  GLN A 589      8.457 -13.211   4.840  1.00 37.03           C
ATOM    865  CG  GLN A 589      7.561 -13.284   3.632  1.00 38.23           C
ATOM    868  CD  GLN A 589      6.518 -14.388   3.787  1.00 41.94           C
ATOM    869  OE1 GLN A 589      5.522 -14.216   4.506  1.00 45.87           O
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ATOM    870  NE2 GLN A 589      6.749 -15.526   3.139  1.00 41.94           N
ATOM    873  C   GLN A 589      9.625 -11.791   6.541  1.00 36.91           C
ATOM    874  O   GLN A 589      9.285 -12.617   7.379  1.00 36.83           O
ATOM    875  N   MET A 590     10.466 -10.816   6.826  1.00 37.58           N
ATOM    877  CA  MET A 590     10.983 -10.659   8.180  1.00 37.70           C
ATOM    879  CB  MET A 590     12.018  -9.572   8.217  1.00 37.85           C
ATOM    882  CG  MET A 590     13.186  -9.849   7.341  1.00 38.04           C
ATOM    885  SD  MET A 590     14.419  -8.644   7.693  1.00 35.14           S
ATOM    886  CE  MET A 590     15.717  -9.257   6.669  1.00 35.41           C
ATOM    890  C   MET A 590      9.872 -10.288   9.128  1.00 37.86           C
ATOM    891  O   MET A 590      9.052  -9.443   8.813  1.00 38.38           O
ATOM    892  N   LYS A 591      9.837 -10.945  10.279  1.00 38.15           N
ATOM    894  CA  LYS A 591      8.843 -10.663  11.296  1.00 38.30           C
ATOM    896  CB  LYS A 591      8.629 -11.889  12.170  1.00 38.99           C
ATOM    899  CG  LYS A 591      7.890 -13.003  11.411  1.00 41.53           C
ATOM    902  CD  LYS A 591      7.746 -14.298  12.210  1.00 44.02           C
ATOM    905  CE  LYS A 591      7.783 -15.538  11.299  1.00 45.54           C
ATOM    908  NZ  LYS A 591      7.982 -16.789  12.087  1.00 46.91           N
ATOM    912  C   LYS A 591      9.345  -9.496  12.115  1.00 37.85           C
ATOM    913  O   LYS A 591     10.519  -9.454  12.465  1.00 37.03           O
ATOM    914  N   HIS A 592      8.463  -8.544  12.402  1.00 37.27           N
ATOM    916  CA  HIS A 592      8.871  -7.309  13.057  1.00 37.50           C
ATOM    918  CB  HIS A 592      7.672  -6.412  13.363  1.00 37.66           C
ATOM    921  CG  HIS A 592      8.051  -5.064  13.905  1.00 38.68           C
ATOM    922  ND1 HIS A 592      8.522  -4.046  13.101  1.00 40.07           N
ATOM    924  CE1 HIS A 592      8.765  -2.976  13.842  1.00 40.15           C
ATOM    926  NE2 HIS A 592      8.478  -3.265  15.100  1.00 39.72           N
ATOM    928  CD2 HIS A 592      8.041  -4.571  15.168  1.00 40.44           C
ATOM    930  C   HIS A 592      9.649  -7.558  14.344  1.00 36.99           C
ATOM    931  O   HIS A 592     10.750  -7.036  14.515  1.00 37.35           O
ATOM    932  N   GLU A 593      9.082  -8.355  15.239  1.00 36.02           N
ATOM    934  CA  GLU A 593      9.681  -8.581  16.550  1.00 35.63           C
ATOM    936  CB  GLU A 593      8.704  -9.334  17.453  1.00 35.97           C
ATOM    939  CG  GLU A 593      9.338  -9.921  18.702  1.00 37.96           C
ATOM    942  CD  GLU A 593      8.332 -10.124  19.810  1.00 41.88           C
ATOM    943  OE1 GLU A 593      7.598 -11.140  19.759  1.00 41.81           O
ATOM    944  OE2 GLU A 593      8.282  -9.260  20.718  1.00 44.16           O
ATOM    945  C   GLU A 593     11.018  -9.329  16.471  1.00 34.35           C
ATOM    946  O   GLU A 593     11.894  -9.161  17.332  1.00 34.16           O
ATOM    947  N   VAL A 594     11.153 -10.162  15.448  1.00 33.24           N
ATOM    949  CA  VAL A 594     12.380 -10.908  15.190  1.00 32.19           C
ATOM    951  CB  VAL A 594     12.153 -12.063  14.144  1.00 31.94           C
ATOM    953  CG1 VAL A 594     13.463 -12.690  13.701  1.00 32.10           C
ATOM    957  CG2 VAL A 594     11.245 -13.152  14.729  1.00 31.70           C
ATOM    961  C   VAL A 594     13.473  -9.950  14.727  1.00 31.67           C
ATOM    962  O   VAL A 594     14.589 -10.006  15.222  1.00 30.91           O
ATOM    963  N   LEU A 595     13.139  -9.062  13.796  1.00 31.23           N
ATOM    965  CA  LEU A 595     14.072  -8.051  13.343  1.00 31.69           C
ATOM    967  CB  LEU A 595     13.452  -7.192  12.247  1.00 32.15           C
ATOM    970  CG  LEU A 595     14.354  -6.039  11.779  1.00 32.94           C
ATOM    972  CD1 LEU A 595     15.661  -6.583  11.252  1.00 34.64           C
ATOM    976  CD2 LEU A 595     13.628  -5.216  10.716  1.00 33.64           C
ATOM    980  C   LEU A 595     14.510  -7.159  14.509  1.00 31.38           C
ATOM    981  O   LEU A 595     15.700  -6.873  14.654  1.00 30.61           O
ATOM    982  N   CYS A 596     13.551  -6.749  15.338  1.00 30.97           N
ATOM    984  CA  CYS A 596     13.847  -5.941  16.527  1.00 31.39           C
ATOM    986  CB  CYS A 596     12.575  -5.557  17.280  1.00 31.32           C
ATOM    989  SG  CYS A 596     11.599  -4.292  16.461  1.00 34.15           S
ATOM    990  C   CYS A 596     14.798  -6.652  17.481  1.00 30.86           C
ATOM    991  O   CYS A 596     15.764  -6.037  17.972  1.00 31.85           O
ATOM    992  N   ARG A 597     14.542  -7.939  17.716  1.00 30.17           N
ATOM    994  CA  ARG A 597     15.348  -8.752  18.630  1.00 29.68           C
ATOM    996  CB  ARG A 597     14.703 -10.111  18.898  1.00 29.75           C
ATOM    999  CG  ARG A 597     15.395 -10.929  19.982  1.00 30.24           C
ATOM   1002  CD  ARG A 597     14.737 -12.269  20.280  1.00 31.74           C
ATOM   1005  NE  ARG A 597     13.494 -12.168  21.053  1.00 32.07           N
ATOM   1007  CZ  ARG A 597     12.256 -12.327  20.567  1.00 34.88           C
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ATOM   1008  NH1  ARG A 597    12.021 -12.579  19.272  1.00 35.26          N
ATOM   1011  NH2  ARG A 597    11.223 -12.235  21.392  1.00 36.29          N
ATOM   1014  C    ARG A 597    16.742  -8.967  18.079  1.00 29.40          C
ATOM   1015  O    ARG A 597    17.702  -8.986  18.837  1.00 28.15          O
ATOM   1016  N    TRP A 598    16.835  -9.163  16.764  1.00 29.37          N
ATOM   1018  CA   TRP A 598    18.120  -9.347  16.095  1.00 28.82          C
ATOM   1020  CB   TRP A 598    17.928  -9.739  14.621  1.00 29.27          C
ATOM   1023  CG   TRP A 598    19.248  -9.848  13.909  1.00 28.69          C
ATOM   1024  CD1  TRP A 598    20.113 -10.900  13.937  1.00 29.65          C
ATOM   1026  NE1  TRP A 598    21.242 -10.601  13.207  1.00 31.90          N
ATOM   1028  CE2  TRP A 598    21.118  -9.332  12.699  1.00 29.14          C
ATOM   1029  CD2  TRP A 598    19.879  -8.827  13.131  1.00 29.44          C
ATOM   1030  CE3  TRP A 598    19.512  -7.531  12.749  1.00 29.44          C
ATOM   1032  CZ3  TRP A 598    20.357  -6.821  11.944  1.00 29.79          C
ATOM   1034  CH2  TRP A 598    21.589  -7.355  11.540  1.00 29.29          C
ATOM   1036  CZ2  TRP A 598    21.971  -8.609  11.894  1.00 28.46          C
ATOM   1038  C    TRP A 598    18.979  -8.087  16.215  1.00 28.83          C
ATOM   1039  O    TRP A 598    20.137  -8.164  16.601  1.00 29.05          O
ATOM   1040  N    ILE A 599    18.397  -6.929  15.928  1.00 28.80          N
ATOM   1042  CA   ILE A 599    19.080  -5.646  16.078  1.00 28.57          C
ATOM   1044  CB   ILE A 599    18.170  -4.467  15.670  1.00 28.39          C
ATOM   1046  CG1  ILE A 599    17.855  -4.500  14.172  1.00 29.63          C
ATOM   1049  CD1  ILE A 599    16.764  -3.554  13.747  1.00 30.52          C
ATOM   1053  CG2  ILE A 599    18.860  -3.137  16.006  1.00 30.68          C
ATOM   1057  C    ILE A 599    19.602  -5.451  17.495  1.00 28.27          C
ATOM   1058  O    ILE A 599    20.755  -5.054  17.680  1.00 28.75          O
ATOM   1059  N    LEU A 600    18.760  -5.742  18.482  1.00 27.81          N
ATOM   1061  CA   LEU A 600    19.121  -5.596  19.879  1.00 27.28          C
ATOM   1063  CB   LEU A 600    17.880  -5.666  20.769  1.00 27.30          C
ATOM   1066  CG   LEU A 600    16.934  -4.451  20.673  1.00 27.61          C
ATOM   1068  CD1  LEU A 600    15.643  -4.734  21.372  1.00 27.09          C
ATOM   1072  CD2  LEU A 600    17.568  -3.184  21.253  1.00 28.63          C
ATOM   1076  C    LEU A 600    20.187  -6.617  20.327  1.00 27.27          C
ATOM   1077  O    LEU A 600    21.028  -6.290  21.161  1.00 26.24          O
ATOM   1078  N    SER A 601    20.173  -7.826  19.767  1.00 26.57          N
ATOM   1080  CA   SER A 601    21.213  -8.837  20.051  1.00 26.67          C
ATOM   1082  CB   SER A 601    20.821 -10.215  19.479  1.00 26.15          C
ATOM   1085  OG   SER A 601    19.720 -10.813  20.166  1.00 25.67          O
ATOM   1087  C    SER A 601    22.573  -8.411  19.453  1.00 26.88          C
ATOM   1088  O    SER A 601    23.628  -8.653  20.017  1.00 26.05          O
ATOM   1089  N    VAL A 602    22.549  -7.797  18.283  1.00 27.58          N
ATOM   1091  CA   VAL A 602    23.780  -7.352  17.656  1.00 27.77          C
ATOM   1093  CB   VAL A 602    23.508  -6.832  16.231  1.00 27.87          C
ATOM   1095  CG1  VAL A 602    24.653  -5.965  15.737  1.00 29.12          C
ATOM   1099  CG2  VAL A 602    23.240  -7.994  15.253  1.00 28.50          C
ATOM   1103  C    VAL A 602    24.377  -6.242  18.541  1.00 28.34          C
ATOM   1104  O    VAL A 602    25.548  -6.286  18.926  1.00 27.60          O
ATOM   1105  N    LYS A 603    23.556  -5.254  18.872  1.00 28.70          N
ATOM   1107  CA   LYS A 603    24.003  -4.148  19.717  1.00 29.38          C
ATOM   1109  CB   LYS A 603    22.872  -3.155  19.943  1.00 29.42          C
ATOM   1112  CG   LYS A 603    23.262  -1.941  20.782  1.00 31.62          C
ATOM   1115  CD   LYS A 603    22.194  -0.897  20.738  1.00 32.61          C
ATOM   1118  CE   LYS A 603    21.027  -1.283  21.599  1.00 35.34          C
ATOM   1121  NZ   LYS A 603    19.869  -0.628  21.043  1.00 39.66          N
ATOM   1125  C    LYS A 603    24.540  -4.661  21.056  1.00 29.88          C
ATOM   1126  O    LYS A 603    25.572  -4.211  21.514  1.00 30.29          O
ATOM   1127  N    LYS A 604    23.850  -5.613  21.671  1.00 30.04          N
ATOM   1129  CA   LYS A 604    24.324  -6.207  22.923  1.00 30.95          C
ATOM   1131  CB   LYS A 604    23.352  -7.286  23.424  1.00 30.85          C
ATOM   1134  CG   LYS A 604    22.126  -6.725  24.009  1.00 35.33          C
ATOM   1137  CD   LYS A 604    21.072  -7.789  24.380  1.00 38.98          C
ATOM   1140  CE   LYS A 604    19.936  -7.184  25.234  1.00 40.81          C
ATOM   1143  NZ   LYS A 604    20.026  -5.712  25.462  1.00 41.19          N
ATOM   1147  C    LYS A 604    25.708  -6.837  22.784  1.00 30.29          C
ATOM   1148  O    LYS A 604    26.537  -6.703  23.665  1.00 29.81          O
ATOM   1149  N    ASN A 605    25.928  -7.572  21.699  1.00 30.06          N
ATOM   1151  CA   ASN A 605    27.205  -8.263  21.505  1.00 30.33          C
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ATOM   1153  CB  ASN A 605     27.054  -9.347  20.438  1.00 30.19           C
ATOM   1156  CG  ASN A 605     26.464 -10.614  21.027  1.00 31.60           C
ATOM   1157  OD1 ASN A 605     27.171 -11.381  21.662  1.00 34.48           O
ATOM   1158  ND2 ASN A 605     25.154 -10.782  20.910  1.00 29.75           N
ATOM   1161  C   ASN A 605     28.393  -7.351  21.222  1.00 30.31           C
ATOM   1162  O   ASN A 605     29.541  -7.779  21.325  1.00 29.93           O
ATOM   1163  N   TYR A 606     28.096  -6.101  20.864  1.00 30.77           N
ATOM   1165  CA  TYR A 606     29.094  -5.027  20.714  1.00 31.16           C
ATOM   1167  CB  TYR A 606     28.524  -3.899  19.833  1.00 30.63           C
ATOM   1170  CG  TYR A 606     28.757  -4.129  18.378  1.00 31.32           C
ATOM   1171  CD1 TYR A 606     30.042  -4.101  17.865  1.00 31.76           C
ATOM   1173  CE1 TYR A 606     30.287  -4.334  16.537  1.00 32.42           C
ATOM   1175  CZ  TYR A 606     29.238  -4.600  15.683  1.00 31.75           C
ATOM   1176  OH  TYR A 606     29.521  -4.835  14.353  1.00 31.75           O
ATOM   1178  CE2 TYR A 606     27.950  -4.623  16.154  1.00 30.16           C
ATOM   1180  CD2 TYR A 606     27.713  -4.394  17.511  1.00 30.01           C
ATOM   1182  C   TYR A 606     29.553  -4.389  22.023  1.00 31.98           C
ATOM   1183  O   TYR A 606     30.468  -3.594  21.996  1.00 31.56           O
ATOM   1184  N   ARG A 607     28.898  -4.693  23.142  1.00 33.28           N
ATOM   1186  CA  ARG A 607     29.146  -4.016  24.429  1.00 35.02           C
ATOM   1188  CB  ARG A 607     28.336  -4.684  25.551  1.00 35.04           C
ATOM   1191  CG  ARG A 607     26.959  -4.155  25.727  1.00 37.70           C
ATOM   1194  CD  ARG A 607     26.294  -4.678  26.991  1.00 39.36           C
ATOM   1197  NE  ARG A 607     24.844  -4.703  26.835  1.00 42.67           N
ATOM   1199  CZ  ARG A 607     24.010  -5.430  27.581  1.00 43.78           C
ATOM   1200  NH1 ARG A 607     24.466  -6.224  28.552  1.00 43.30           N
ATOM   1203  NH2 ARG A 607     22.700  -5.370  27.345  1.00 45.74           N
ATOM   1206  C   ARG A 607     30.577  -3.987  24.939  1.00 35.46           C
ATOM   1207  O   ARG A 607     31.006  -3.004  25.514  1.00 35.88           O
ATOM   1208  N   LYS A 608     31.290  -5.096  24.824  1.00 36.93           N
ATOM   1210  CA  LYS A 608     32.666  -5.142  25.335  1.00 38.15           C
ATOM   1212  CB  LYS A 608     33.068  -6.578  25.711  1.00 38.79           C
ATOM   1215  CG  LYS A 608     32.304  -7.171  26.889  1.00 39.97           C
ATOM   1218  CD  LYS A 608     33.209  -7.466  28.089  1.00 41.39           C
ATOM   1221  CE  LYS A 608     32.865  -8.784  28.755  1.00 41.54           C
ATOM   1224  NZ  LYS A 608     33.799  -9.043  29.896  1.00 42.55           N
ATOM   1228  C   LYS A 608     33.696  -4.570  24.353  1.00 37.89           C
ATOM   1229  O   LYS A 608     34.874  -4.506  24.682  1.00 38.34           O
ATOM   1230  N   ASN A 609     33.274  -4.159  23.161  1.00 37.47           N
ATOM   1232  CA  ASN A 609     34.227  -3.613  22.187  1.00 37.88           C
ATOM   1234  CB  ASN A 609     33.629  -3.620  20.788  1.00 37.01           C
ATOM   1237  CG  ASN A 609     33.576  -5.002  20.180  1.00 37.42           C
ATOM   1238  OD1 ASN A 609     33.620  -6.011  20.880  1.00 38.35           O
ATOM   1239  ND2 ASN A 609     33.471  -5.053  18.871  1.00 32.99           N
ATOM   1242  C   ASN A 609     34.701  -2.169  22.517  1.00 38.36           C
ATOM   1243  O   ASN A 609     33.946  -1.387  23.070  1.00 36.98           O
ATOM   1244  N   VAL A 610     35.930  -1.824  22.091  1.00 39.12           N
ATOM   1246  CA  VAL A 610     36.442  -0.452  22.196  1.00 39.82           C
ATOM   1248  CB  VAL A 610     37.884  -0.297  21.640  1.00 41.13           C
ATOM   1250  CG1 VAL A 610     38.517   1.049  22.100  1.00 40.50           C
ATOM   1254  CG2 VAL A 610     38.757  -1.484  22.068  1.00 43.89           C
ATOM   1258  C   VAL A 610     35.551   0.386  21.319  1.00 39.18           C
ATOM   1259  O   VAL A 610     34.918  -0.153  20.399  1.00 38.76           O
ATOM   1260  N   ALA A 611     35.527   1.687  21.572  1.00 37.98           N
ATOM   1262  CA  ALA A 611     34.487   2.544  21.029  1.00 37.77           C
ATOM   1264  CB  ALA A 611     34.527   3.959  21.638  1.00 38.26           C
ATOM   1268  C   ALA A 611     34.569   2.609  19.562  1.00 37.90           C
ATOM   1269  O   ALA A 611     33.529   2.668  18.886  1.00 39.09           O
ATOM   1270  N   TYR A 612     35.789   2.555  19.026  1.00 37.10           N
ATOM   1272  CA  TYR A 612     35.907   2.589  17.593  1.00 36.21           C
ATOM   1274  CB  TYR A 612     37.279   3.132  17.144  1.00 35.83           C
ATOM   1277  CG  TYR A 612     38.548   2.537  17.706  1.00 31.94           C
ATOM   1278  CD1 TYR A 612     39.280   3.193  18.678  1.00 30.63           C
ATOM   1280  CE1 TYR A 612     40.486   2.685  19.128  1.00 29.64           C
ATOM   1282  CZ  TYR A 612     40.997   1.524  18.566  1.00 30.05           C
ATOM   1283  OH  TYR A 612     42.199   0.984  18.976  1.00 27.48           O
ATOM   1285  CE2 TYR A 612     40.280   0.859  17.593  1.00 28.47           C
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ATOM    1287  CD2 TYR A 612      39.090    1.383   17.152  1.00 31.42           C
ATOM    1289  C   TYR A 612      35.504    1.301   16.870  1.00 36.03           C
ATOM    1290  O   TYR A 612      35.376    1.316   15.645  1.00 36.29           O
ATOM    1291  N   HIS A 613      35.285    0.190   17.585  1.00 35.22           N
ATOM    1293  CA  HIS A 613      34.680   -1.018   16.950  1.00 34.43           C
ATOM    1295  CB  HIS A 613      35.592   -2.255   17.069  1.00 34.90           C
ATOM    1298  CG  HIS A 613      36.922   -2.105   16.400  1.00 36.25           C
ATOM    1299  ND1 HIS A 613      37.063   -1.612   15.113  1.00 35.47           N
ATOM    1301  CE1 HIS A 613      38.348   -1.602   14.792  1.00 38.87           C
ATOM    1303  NE2 HIS A 613      39.038   -2.116   15.798  1.00 37.92           N
ATOM    1305  CD2 HIS A 613      38.168   -2.442   16.818  1.00 37.07           C
ATOM    1307  C   HIS A 613      33.342   -1.367   17.591  1.00 33.82           C
ATOM    1308  O   HIS A 613      32.970   -2.551   17.697  1.00 32.40           O
ATOM    1309  N   ASN A 614      32.651   -0.328   18.049  1.00 33.04           N
ATOM    1311  CA  ASN A 614      31.435   -0.486   18.822  1.00 32.15           C
ATOM    1313  CB  ASN A 614      31.384    0.536   19.980  1.00 31.90           C
ATOM    1316  CG  ASN A 614      31.107    1.966   19.513  1.00 32.57           C
ATOM    1317  OD1 ASN A 614      30.893    2.227   18.324  1.00 34.59           O
ATOM    1318  ND2 ASN A 614      31.137    2.895   20.446  1.00 29.51           N
ATOM    1321  C   ASN A 614      30.202   -0.410   17.902  1.00 31.23           C
ATOM    1322  O   ASN A 614      30.323   -0.220   16.664  1.00 29.60           O
ATOM    1323  N   TRP A 615      29.035   -0.579   18.517  1.00 30.30           N
ATOM    1325  CA  TRP A 615      27.760   -0.531   17.812  1.00 30.70           C
ATOM    1327  CB  TRP A 615      26.585   -0.716   18.788  1.00 30.92           C
ATOM    1330  CG  TRP A 615      25.286   -0.217   18.237  1.00 31.99           C
ATOM    1331  CD1 TRP A 615      24.620    0.880   18.640  1.00 32.83           C
ATOM    1333  NE1 TRP A 615      23.474    1.035   17.900  1.00 33.36           N
ATOM    1335  CE2 TRP A 615      23.378    0.012   17.001  1.00 31.11           C
ATOM    1336  CD2 TRP A 615      24.506   -0.802   17.188  1.00 30.42           C
ATOM    1337  CE3 TRP A 615      24.647   -1.937   16.381  1.00 32.01           C
ATOM    1339  CZ3 TRP A 615      23.658   -2.215   15.428  1.00 30.94           C
ATOM    1341  CH2 TRP A 615      22.556   -1.376   15.269  1.00 31.69           C
ATOM    1343  CZ2 TRP A 615      22.384   -0.270   16.057  1.00 31.08           C
ATOM    1345  C   TRP A 615      27.583    0.755   17.002  1.00 30.51           C
ATOM    1346  O   TRP A 615      27.164    0.694   15.873  1.00 31.12           O
ATOM    1347  N   ARG A 616      27.963    1.902   17.542  1.00 30.28           N
ATOM    1349  CA  ARG A 616      27.832    3.144   16.785  1.00 31.43           C
ATOM    1351  CB  ARG A 616      28.192    4.379   17.621  1.00 31.30           C
ATOM    1354  CG  ARG A 616      27.202    4.659   18.759  1.00 33.10           C
ATOM    1357  CD  ARG A 616      25.719    4.547   18.363  1.00 35.82           C
ATOM    1360  NE  ARG A 616      24.840    4.907   19.468  1.00 37.45           N
ATOM    1362  CZ  ARG A 616      24.435    6.147   19.764  1.00 37.21           C
ATOM    1363  NH1 ARG A 616      24.797    7.196   19.031  1.00 35.24           N
ATOM    1366  NH2 ARG A 616      23.641    6.326   20.806  1.00 36.40           N
ATOM    1369  C   ARG A 616      28.647    3.113   15.502  1.00 31.51           C
ATOM    1370  O   ARG A 616      28.148    3.501   14.447  1.00 32.82           O
ATOM    1371  N   HIS A 617      29.875    2.614   15.546  1.00 31.18           N
ATOM    1373  CA  HIS A 617      30.606    2.498   14.312  1.00 30.51           C
ATOM    1375  CB  HIS A 617      32.016    2.029   14.543  1.00 31.02           C
ATOM    1378  CG  HIS A 617      32.728    1.695   13.269  1.00 30.16           C
ATOM    1379  ND1 HIS A 617      33.123    2.656   12.380  1.00 27.25           N
ATOM    1381  CE1 HIS A 617      33.698    2.080   11.344  1.00 31.29           C
ATOM    1383  NE2 HIS A 617      33.608    0.781   11.493  1.00 28.84           N
ATOM    1385  CD2 HIS A 617      33.017    0.511   12.696  1.00 29.49           C
ATOM    1387  C   HIS A 617      29.929    1.577   13.292  1.00 30.71           C
ATOM    1388  O   HIS A 617      29.861    1.885   12.099  1.00 30.52           O
ATOM    1389  N   ALA A 618      29.453    0.440   13.750  1.00 30.71           N
ATOM    1391  CA  ALA A 618      28.829   -0.536   12.861  1.00 30.45           C
ATOM    1393  CB  ALA A 618      28.498   -1.823   13.630  1.00 30.32           C
ATOM    1397  C   ALA A 618      27.565    0.061   12.258  1.00 30.85           C
ATOM    1398  O   ALA A 618      27.290   -0.087   11.071  1.00 31.51           O
ATOM    1399  N   PHE A 619      26.803    0.755   13.093  1.00 31.16           N
ATOM    1401  CA  PHE A 619      25.562    1.393   12.682  1.00 30.81           C
ATOM    1403  CB  PHE A 619      24.853    1.997   13.896  1.00 30.88           C
ATOM    1406  CG  PHE A 619      23.783    2.974   13.526  1.00 30.88           C
ATOM    1407  CD1 PHE A 619      22.616    2.546   12.947  1.00 29.68           C
ATOM    1409  CE1 PHE A 619      21.635    3.459   12.578  1.00 30.57           C
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ATOM	1411	CZ	PHE	A	619	21.850	4.791	12.751	1.00	31.51	C
ATOM	1413	CE2	PHE	A	619	23.019	5.230	13.319	1.00	32.18	C
ATOM	1415	CD2	PHE	A	619	23.986	4.332	13.689	1.00	31.89	C
ATOM	1417	C	PHE	A	619	25.849	2.469	11.616	1.00	31.44	C
ATOM	1418	O	PHE	A	619	25.139	2.536	10.606	1.00	31.44	O
ATOM	1419	N	ASN	A	620	26.896	3.274	11.832	1.00	30.85	N
ATOM	1421	CA	ASN	A	620	27.363	4.262	10.836	1.00	31.22	C
ATOM	1423	CB	ASN	A	620	28.507	5.126	11.395	1.00	31.15	C
ATOM	1426	CG	ASN	A	620	28.010	6.191	12.378	1.00	34.02	C
ATOM	1427	OD1	ASN	A	620	27.080	6.934	12.076	1.00	39.28	O
ATOM	1428	ND2	ASN	A	620	28.657	6.290	13.543	1.00	35.23	N
ATOM	1431	C	ASN	A	620	27.803	3.663	9.498	1.00	31.18	C
ATOM	1432	O	ASN	A	620	27.540	4.239	8.454	1.00	31.47	O
ATOM	1433	N	THR	A	621	28.484	2.524	9.549	1.00	30.75	N
ATOM	1435	CA	THR	A	621	28.868	1.786	8.378	1.00	31.18	C
ATOM	1437	CB	THR	A	621	29.655	0.560	8.788	1.00	31.37	C
ATOM	1439	OG1	THR	A	621	30.820	0.922	9.574	1.00	33.41	O
ATOM	1441	CG2	THR	A	621	30.229	-0.137	7.558	1.00	31.84	C
ATOM	1445	C	THR	A	621	27.614	1.362	7.575	1.00	31.48	C
ATOM	1446	O	THR	A	621	27.571	1.507	6.374	1.00	30.62	O
ATOM	1447	N	ALA	A	622	26.584	0.893	8.266	1.00	32.36	N
ATOM	1449	CA	ALA	A	622	25.315	0.494	7.643	1.00	32.22	C
ATOM	1451	CB	ALA	A	622	24.433	-0.195	8.662	1.00	32.79	C
ATOM	1455	C	ALA	A	622	24.573	1.674	7.064	1.00	32.14	C
ATOM	1456	O	ALA	A	622	24.025	1.562	5.973	1.00	31.93	O
ATOM	1457	N	GLN	A	623	24.567	2.811	7.769	1.00	31.86	N
ATOM	1459	CA	GLN	A	623	23.886	3.999	7.274	1.00	31.61	C
ATOM	1461	CB	GLN	A	623	23.809	5.080	8.332	1.00	31.93	C
ATOM	1464	CG	GLN	A	623	23.074	6.375	7.924	1.00	31.37	C
ATOM	1467	CD	GLN	A	623	23.927	7.312	7.084	1.00	30.66	C
ATOM	1468	OE1	GLN	A	623	25.155	7.428	7.290	1.00	31.16	O
ATOM	1469	NE2	GLN	A	623	23.293	7.974	6.136	1.00	29.92	N
ATOM	1472	C	GLN	A	623	24.554	4.525	6.012	1.00	31.95	C
ATOM	1473	O	GLN	A	623	23.859	4.910	5.061	1.00	32.19	O
ATOM	1474	N	CYS	A	624	25.884	4.503	5.970	1.00	31.30	N
ATOM	1476	CA	CYS	A	624	26.592	4.885	4.759	1.00	31.66	C
ATOM	1478	CB	CYS	A	624	28.100	4.862	4.950	1.00	31.87	C
ATOM	1481	SG	CYS	A	624	29.005	5.620	3.568	1.00	32.91	S
ATOM	1482	C	CYS	A	624	26.217	3.949	3.590	1.00	31.58	C
ATOM	1483	O	CYS	A	624	26.085	4.406	2.473	1.00	30.48	O
ATOM	1484	N	MET	A	625	26.073	2.653	3.862	1.00	31.89	N
ATOM	1486	CA	MET	A	625	25.606	1.680	2.850	1.00	32.02	C
ATOM	1488	CB	MET	A	625	25.611	0.262	3.449	1.00	32.34	C
ATOM	1491	CG	MET	A	625	25.292	-0.885	2.503	1.00	32.88	C
ATOM	1494	SD	MET	A	625	26.447	-1.020	1.189	1.00	35.04	S
ATOM	1495	CE	MET	A	625	27.759	-1.743	1.958	1.00	34.33	C
ATOM	1499	C	MET	A	625	24.216	2.056	2.326	1.00	32.02	C
ATOM	1500	O	MET	A	625	23.997	2.110	1.121	1.00	32.01	O
ATOM	1501	N	PHE	A	626	23.283	2.357	3.228	1.00	32.02	N
ATOM	1503	CA	PHE	A	626	21.938	2.757	2.838	1.00	31.43	C
ATOM	1505	CB	PHE	A	626	21.050	2.992	4.077	1.00	31.75	C
ATOM	1508	CG	PHE	A	626	19.652	3.449	3.755	1.00	31.75	C
ATOM	1509	CD1	PHE	A	626	18.638	2.523	3.525	1.00	30.61	C
ATOM	1511	CE1	PHE	A	626	17.364	2.939	3.231	1.00	32.72	C
ATOM	1513	CZ	PHE	A	626	17.066	4.312	3.168	1.00	32.65	C
ATOM	1515	CE2	PHE	A	626	18.073	5.235	3.404	1.00	33.06	C
ATOM	1517	CD2	PHE	A	626	19.355	4.805	3.689	1.00	31.65	C
ATOM	1519	C	PHE	A	626	22.008	4.016	1.987	1.00	31.60	C
ATOM	1520	O	PHE	A	626	21.331	4.119	0.960	1.00	31.35	O
ATOM	1521	N	ALA	A	627	22.825	4.971	2.409	1.00	31.24	N
ATOM	1523	CA	ALA	A	627	22.931	6.228	1.697	1.00	31.47	C
ATOM	1525	CB	ALA	A	627	23.790	7.240	2.471	1.00	31.67	C
ATOM	1529	C	ALA	A	627	23.520	5.958	0.321	1.00	31.49	C
ATOM	1530	O	ALA	A	627	23.007	6.465	-0.679	1.00	31.23	O
ATOM	1531	N	ALA	A	628	24.569	5.140	0.266	1.00	30.86	N
ATOM	1533	CA	ALA	A	628	25.179	4.800	-1.024	1.00	31.71	C
ATOM	1535	CB	ALA	A	628	26.466	4.007	-0.843	1.00	31.46	C

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ATOM   1539  C    ALA A 628     24.193   4.067  -1.955  1.00 31.94           C
ATOM   1540  O    ALA A 628     24.194   4.311  -3.149  1.00 32.27           O
ATOM   1541  N    LEU A 629     23.305   3.243  -1.401  1.00 32.55           N
ATOM   1543  CA   LEU A 629     22.332   2.515  -2.204  1.00 32.89           C
ATOM   1545  CB   LEU A 629     21.719   1.357  -1.403  1.00 33.12           C
ATOM   1548  CG   LEU A 629     22.663   0.204  -1.000  1.00 34.14           C
ATOM   1550  CD1  LEU A 629     22.092  -0.664   0.149  1.00 34.32           C
ATOM   1554  CD2  LEU A 629     22.978  -0.669  -2.144  1.00 34.96           C
ATOM   1558  C    LEU A 629     21.243   3.455  -2.707  1.00 32.75           C
ATOM   1559  O    LEU A 629     20.725   3.285  -3.820  1.00 32.52           O
ATOM   1560  N    LYS A 630     20.895   4.456  -1.897  1.00 32.92           N
ATOM   1562  CA   LYS A 630     19.762   5.340  -2.192  1.00 32.40           C
ATOM   1564  CB   LYS A 630     18.985   5.661  -0.916  1.00 32.67           C
ATOM   1567  CG   LYS A 630     18.220   4.478  -0.352  1.00 34.05           C
ATOM   1570  CD   LYS A 630     17.077   4.068  -1.296  1.00 35.47           C
ATOM   1573  CE   LYS A 630     16.130   3.083  -0.647  1.00 36.74           C
ATOM   1576  NZ   LYS A 630     14.939   2.850  -1.507  1.00 37.93           N
ATOM   1580  C    LYS A 630     20.199   6.626  -2.881  1.00 32.33           C
ATOM   1581  O    LYS A 630     19.934   6.834  -4.055  1.00 32.10           O
ATOM   1582  N    ALA A 631     20.865   7.502  -2.150  1.00 32.35           N
ATOM   1584  CA   ALA A 631     21.341   8.762  -2.723  1.00 31.80           C
ATOM   1586  CB   ALA A 631     21.944   9.623  -1.632  1.00 32.01           C
ATOM   1590  C    ALA A 631     22.385   8.487  -3.810  1.00 31.40           C
ATOM   1591  O    ALA A 631     22.409   9.145  -4.848  1.00 29.37           O
ATOM   1592  N    GLY A 632     23.249   7.510  -3.547  1.00 31.00           N
ATOM   1594  CA   GLY A 632     24.308   7.152  -4.474  1.00 31.53           C
ATOM   1597  C    GLY A 632     23.842   6.238  -5.599  1.00 31.81           C
ATOM   1598  O    GLY A 632     24.651   5.877  -6.453  1.00 32.08           O
ATOM   1599  N    LYS A 633     22.566   5.842  -5.575  1.00 32.51           N
ATOM   1601  CA   LYS A 633     21.926   5.037  -6.631  1.00 33.28           C
ATOM   1603  CB   LYS A 633     21.631   5.921  -7.843  1.00 34.01           C
ATOM   1606  CG   LYS A 633     20.450   6.887  -7.619  1.00 34.40           C
ATOM   1609  CD   LYS A 633     20.242   7.895  -8.775  1.00 35.77           C
ATOM   1612  CE   LYS A 633     20.244   7.226 -10.165  1.00 36.91           C
ATOM   1615  NZ   LYS A 633     19.667   8.095 -11.259  1.00 34.73           N
ATOM   1619  C    LYS A 633     22.674   3.751  -7.031  1.00 33.99           C
ATOM   1620  O    LYS A 633     22.746   3.386  -8.200  1.00 34.69           O
ATOM   1621  N    ILE A 634     23.227   3.060  -6.039  1.00 34.33           N
ATOM   1623  CA   ILE A 634     23.870   1.772  -6.260  1.00 34.38           C
ATOM   1625  CB   ILE A 634     25.100   1.652  -5.329  1.00 34.92           C
ATOM   1627  CG1  ILE A 634     26.218   2.516  -5.884  1.00 35.10           C
ATOM   1630  CD1  ILE A 634     27.081   2.989  -4.854  1.00 37.95           C
ATOM   1634  CG2  ILE A 634     25.615   0.176  -5.155  1.00 35.62           C
ATOM   1638  C    ILE A 634     22.855   0.623  -6.091  1.00 34.47           C
ATOM   1639  O    ILE A 634     23.134  -0.519  -6.458  1.00 33.68           O
ATOM   1640  N    GLN A 635     21.671   0.943  -5.567  1.00 34.21           N
ATOM   1642  CA   GLN A 635     20.625  -0.049  -5.353  1.00 34.80           C
ATOM   1644  CB   GLN A 635     19.403   0.601  -4.706  1.00 35.03           C
ATOM   1647  CG   GLN A 635     18.209  -0.315  -4.540  1.00 36.49           C
ATOM   1650  CD   GLN A 635     17.084   0.307  -3.721  1.00 39.00           C
ATOM   1651  OE1  GLN A 635     16.900   1.526  -3.705  1.00 39.26           O
ATOM   1652  NE2  GLN A 635     16.318  -0.540  -3.055  1.00 42.25           N
ATOM   1655  C    GLN A 635     20.231  -0.754  -6.670  1.00 35.04           C
ATOM   1656  O    GLN A 635     20.076  -1.975  -6.685  1.00 33.84           O
ATOM   1657  N    ASN A 636     20.085   0.041  -7.741  1.00 35.20           N
ATOM   1659  CA   ASN A 636     19.770  -0.431  -9.090  1.00 36.02           C
ATOM   1661  CB   ASN A 636     19.655   0.738 -10.103  1.00 36.68           C
ATOM   1664  CG   ASN A 636     18.818   1.904  -9.595  1.00 39.99           C
ATOM   1665  OD1  ASN A 636     18.140   1.803  -8.565  1.00 46.78           O
ATOM   1666  ND2  ASN A 636     18.861   3.030 -10.321  1.00 42.53           N
ATOM   1669  C    ASN A 636     20.802  -1.389  -9.666  1.00 35.44           C
ATOM   1670  O    ASN A 636     20.492  -2.168 -10.552  1.00 35.08           O
ATOM   1671  N    LYS A 637     22.038  -1.296  -9.192  1.00 35.20           N
ATOM   1673  CA   LYS A 637     23.119  -2.130  -9.682  1.00 34.87           C
ATOM   1675  CB   LYS A 637     24.436  -1.348  -9.650  1.00 35.40           C
ATOM   1678  CG   LYS A 637     24.484  -0.150 -10.616  1.00 36.49           C
ATOM   1681  CD   LYS A 637     25.718   0.730 -10.327  1.00 38.37           C
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ATOM    1684  CE   LYS A 637      25.539    2.148 -10.877  1.00 38.75           C
ATOM    1687  NZ   LYS A 637      25.609    2.142 -12.360  1.00 38.23           N
ATOM    1691  C    LYS A 637      23.291   -3.435  -8.913  1.00 34.18           C
ATOM    1692  O    LYS A 637      24.107   -4.258  -9.304  1.00 33.84           O
ATOM    1693  N    LEU A 638      22.542   -3.628  -7.834  1.00 33.19           N
ATOM    1695  CA   LEU A 638      22.712   -4.805  -6.979  1.00 32.72           C
ATOM    1697  CB   LEU A 638      23.186   -4.369  -5.581  1.00 32.57           C
ATOM    1700  CG   LEU A 638      24.543   -3.658  -5.477  1.00 32.78           C
ATOM    1702  CD1  LEU A 638      24.915   -3.512  -4.038  1.00 33.08           C
ATOM    1706  CD2  LEU A 638      25.653   -4.387  -6.192  1.00 34.28           C
ATOM    1710  C    LEU A 638      21.423   -5.635  -6.858  1.00 32.44           C
ATOM    1711  O    LEU A 638      20.320   -5.133  -7.095  1.00 32.46           O
ATOM    1712  N    THR A 639      21.558   -6.904  -6.477  1.00 31.41           N
ATOM    1714  CA   THR A 639      20.383   -7.763  -6.278  1.00 31.03           C
ATOM    1716  CB   THR A 639      20.717   -9.246  -6.548  1.00 30.99           C
ATOM    1718  OG1  THR A 639      21.643   -9.717  -5.552  1.00 29.09           O
ATOM    1720  CG2  THR A 639      21.409   -9.440  -7.920  1.00 30.02           C
ATOM    1724  C    THR A 639      19.885   -7.644  -4.848  1.00 30.57           C
ATOM    1725  O    THR A 639      20.586   -7.138  -4.004  1.00 30.89           O
ATOM    1726  N    ASP A 640      18.691   -8.158  -4.585  1.00 30.85           N
ATOM    1728  CA   ASP A 640      18.121   -8.202  -3.245  1.00 31.53           C
ATOM    1730  CB   ASP A 640      16.742   -8.853  -3.244  1.00 32.21           C
ATOM    1733  CG   ASP A 640      15.663   -7.950  -3.781  1.00 36.04           C
ATOM    1734  OD1  ASP A 640      15.767   -6.715  -3.599  1.00 38.91           O
ATOM    1735  OD2  ASP A 640      14.668   -8.407  -4.387  1.00 41.38           O
ATOM    1736  C    ASP A 640      18.997   -8.962  -2.263  1.00 31.29           C
ATOM    1737  O    ASP A 640      19.163   -8.503  -1.146  1.00 31.11           O
ATOM    1738  N    LEU A 641      19.533  -10.115  -2.665  1.00 31.09           N
ATOM    1740  CA   LEU A 641      20.416  -10.897  -1.787  1.00 31.26           C
ATOM    1742  CB   LEU A 641      20.779  -12.249  -2.421  1.00 31.33           C
ATOM    1745  CG   LEU A 641      19.675  -13.291  -2.564  1.00 30.70           C
ATOM    1747  CD1  LEU A 641      20.271  -14.613  -3.042  1.00 30.42           C
ATOM    1751  CD2  LEU A 641      18.875  -13.482  -1.261  1.00 29.87           C
ATOM    1755  C    LEU A 641      21.685  -10.134  -1.449  1.00 30.81           C
ATOM    1756  O    LEU A 641      22.119  -10.146  -0.312  1.00 31.86           O
ATOM    1757  N    GLU A 642      22.259   -9.449  -2.431  1.00 30.40           N
ATOM    1759  CA   GLU A 642      23.483   -8.674  -2.225  1.00 30.27           C
ATOM    1761  CB   GLU A 642      24.032   -8.166  -3.558  1.00 30.21           C
ATOM    1764  CG   GLU A 642      24.558   -9.287  -4.455  1.00 30.03           C
ATOM    1767  CD   GLU A 642      24.788   -8.881  -5.910  1.00 32.15           C
ATOM    1768  OE1  GLU A 642      24.321   -7.805  -6.335  1.00 33.95           O
ATOM    1769  OE2  GLU A 642      25.416   -9.657  -6.666  1.00 32.81           O
ATOM    1770  C    GLU A 642      23.245   -7.528  -1.250  1.00 30.47           C
ATOM    1771  O    GLU A 642      24.048   -7.293  -0.342  1.00 30.71           O
ATOM    1772  N    ILE A 643      22.099   -6.873  -1.401  1.00 30.77           N
ATOM    1774  CA   ILE A 643      21.713   -5.760  -0.569  1.00 30.94           C
ATOM    1776  CB   ILE A 643      20.501   -5.040  -1.122  1.00 30.64           C
ATOM    1778  CG1  ILE A 643      20.882   -4.330  -2.424  1.00 31.48           C
ATOM    1781  CD1  ILE A 643      19.691   -3.841  -3.235  1.00 30.95           C
ATOM    1785  CG2  ILE A 643      19.980   -4.033  -0.117  1.00 31.85           C
ATOM    1789  C    ILE A 643      21.482   -6.207   0.866  1.00 31.22           C
ATOM    1790  O    ILE A 643      21.996   -5.568   1.776  1.00 30.90           O
ATOM    1791  N    LEU A 644      20.753   -7.302   1.048  1.00 30.89           N
ATOM    1793  CA   LEU A 644      20.533   -7.934   2.357  1.00 31.35           C
ATOM    1795  CB   LEU A 644      19.684   -9.204   2.158  1.00 31.85           C
ATOM    1798  CG   LEU A 644      19.344  -10.070   3.376  1.00 32.51           C
ATOM    1800  CD1  LEU A 644      18.561   -9.281   4.419  1.00 31.68           C
ATOM    1804  CD2  LEU A 644      18.557  -11.302   2.924  1.00 34.35           C
ATOM    1808  C    LEU A 644      21.837   -8.298   3.053  1.00 31.35           C
ATOM    1809  O    LEU A 644      22.039   -7.976   4.214  1.00 32.00           O
ATOM    1810  N    ALA A 645      22.746   -8.922   2.320  1.00 30.91           N
ATOM    1812  CA   ALA A 645      23.987   -9.374   2.897  1.00 31.28           C
ATOM    1814  CB   ALA A 645      24.735  -10.286   1.944  1.00 30.79           C
ATOM    1818  C    ALA A 645      24.864   -8.181   3.254  1.00 31.60           C
ATOM    1819  O    ALA A 645      25.548   -8.234   4.244  1.00 32.24           O
ATOM    1820  N    LEU A 646      24.854   -7.138   2.431  1.00 31.70           N
ATOM    1822  CA   LEU A 646      25.726   -5.985   2.633  1.00 32.69           C
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ATOM   1824  CB  LEU A 646      25.701  -5.004   1.465  1.00 33.10           C
ATOM   1827  CG  LEU A 646      26.465  -5.375   0.202  1.00 36.19           C
ATOM   1829  CD1 LEU A 646      25.967  -4.482  -0.948  1.00 37.84           C
ATOM   1833  CD2 LEU A 646      27.950  -5.267   0.411  1.00 37.50           C
ATOM   1837  C   LEU A 646      25.291  -5.242   3.861  1.00 32.76           C
ATOM   1838  O   LEU A 646      26.122  -4.810   4.627  1.00 32.57           O
ATOM   1839  N   LEU A 647      23.985  -5.094   4.035  1.00 33.13           N
ATOM   1841  CA  LEU A 647      23.457  -4.468   5.237  1.00 33.42           C
ATOM   1843  CB  LEU A 647      21.947  -4.256   5.101  1.00 33.13           C
ATOM   1846  CG  LEU A 647      21.357  -3.326   6.155  1.00 34.76           C
ATOM   1848  CD1 LEU A 647      22.086  -1.969   6.182  1.00 34.70           C
ATOM   1852  CD2 LEU A 647      19.850  -3.117   5.959  1.00 35.72           C
ATOM   1856  C   LEU A 647      23.815  -5.284   6.509  1.00 33.49           C
ATOM   1857  O   LEU A 647      24.288  -4.735   7.519  1.00 33.49           O
ATOM   1858  N   ILE A 648      23.657  -6.596   6.450  1.00 33.09           N
ATOM   1860  CA  ILE A 648      23.917  -7.413   7.618  1.00 32.95           C
ATOM   1862  CB  ILE A 648      23.412  -8.832   7.423  1.00 32.70           C
ATOM   1864  CG1 ILE A 648      21.876  -8.857   7.471  1.00 32.59           C
ATOM   1867  CD1 ILE A 648      21.271 -10.103   6.822  1.00 33.18           C
ATOM   1871  CG2 ILE A 648      24.015  -9.759   8.477  1.00 33.03           C
ATOM   1875  C   ILE A 648      25.405  -7.411   7.965  1.00 33.03           C
ATOM   1876  O   ILE A 648      25.755  -7.312   9.138  1.00 32.99           O
ATOM   1877  N   ALA A 649      26.252  -7.529   6.943  1.00 32.46           N
ATOM   1879  CA  ALA A 649      27.694  -7.441   7.091  1.00 32.19           C
ATOM   1881  CB  ALA A 649      28.376  -7.690   5.747  1.00 31.91           C
ATOM   1885  C   ALA A 649      28.166  -6.112   7.683  1.00 31.99           C
ATOM   1886  O   ALA A 649      28.959  -6.097   8.603  1.00 31.02           O
ATOM   1887  N   ALA A 650      27.667  -4.998   7.162  1.00 32.64           N
ATOM   1889  CA  ALA A 650      28.030  -3.672   7.686  1.00 32.16           C
ATOM   1891  CB  ALA A 650      27.265  -2.592   6.957  1.00 32.87           C
ATOM   1895  C   ALA A 650      27.754  -3.599   9.167  1.00 31.97           C
ATOM   1896  O   ALA A 650      28.619  -3.171   9.962  1.00 31.46           O
ATOM   1897  N   LEU A 651      26.582  -4.081   9.556  1.00 31.43           N
ATOM   1899  CA  LEU A 651      26.159  -4.076  10.963  1.00 31.21           C
ATOM   1901  CB  LEU A 651      24.673  -4.404  11.083  1.00 31.32           C
ATOM   1904  CG  LEU A 651      23.742  -3.326  10.542  1.00 32.12           C
ATOM   1906  CD1 LEU A 651      22.344  -3.843  10.355  1.00 32.83           C
ATOM   1910  CD2 LEU A 651      23.743  -2.125  11.438  1.00 32.23           C
ATOM   1914  C   LEU A 651      26.957  -5.047  11.850  1.00 31.12           C
ATOM   1915  O   LEU A 651      27.210  -4.751  13.011  1.00 29.74           O
ATOM   1916  N   SER A 652      27.396  -6.167  11.277  1.00 31.07           N
ATOM   1918  CA  SER A 652      28.029  -7.252  12.038  1.00 31.40           C
ATOM   1920  CB  SER A 652      27.488  -8.590  11.545  1.00 31.66           C
ATOM   1923  OG  SER A 652      26.075  -8.596  11.563  1.00 33.22           O
ATOM   1925  C   SER A 652      29.551  -7.369  11.982  1.00 30.56           C
ATOM   1926  O   SER A 652      30.117  -8.177  12.697  1.00 28.98           O
ATOM   1927  N   HIS A 653      30.212  -6.586  11.144  1.00 30.93           N
ATOM   1929  CA  HIS A 653      31.583  -6.901  10.760  1.00 31.59           C
ATOM   1931  CB  HIS A 653      32.037  -6.037   9.578  1.00 31.74           C
ATOM   1934  CG  HIS A 653      32.262  -4.609   9.935  1.00 32.86           C
ATOM   1935  ND1 HIS A 653      31.236  -3.705  10.043  1.00 34.83           N
ATOM   1937  CE1 HIS A 653      31.724  -2.526  10.380  1.00 33.06           C
ATOM   1939  NE2 HIS A 653      33.035  -2.632  10.474  1.00 34.35           N
ATOM   1941  CD2 HIS A 653      33.397  -3.922  10.205  1.00 34.04           C
ATOM   1943  C   HIS A 653      32.610  -6.765  11.900  1.00 31.77           C
ATOM   1944  O   HIS A 653      33.694  -7.343  11.786  1.00 31.53           O
ATOM   1945  N   ASP A 654      32.287  -6.016  12.967  1.00 31.02           N
ATOM   1947  CA  ASP A 654      33.203  -5.869  14.110  1.00 32.06           C
ATOM   1949  CB  ASP A 654      33.267  -4.406  14.541  1.00 31.95           C
ATOM   1952  CG  ASP A 654      34.342  -3.613  13.798  1.00 34.24           C
ATOM   1953  OD1 ASP A 654      35.199  -4.243  13.103  1.00 32.30           O
ATOM   1954  OD2 ASP A 654      34.419  -2.361  13.876  1.00 34.59           O
ATOM   1955  C   ASP A 654      32.867  -6.772  15.296  1.00 33.06           C
ATOM   1956  O   ASP A 654      33.422  -6.624  16.379  1.00 31.77           O
ATOM   1957  N   LEU A 655      31.933  -7.703  15.108  1.00 34.41           N
ATOM   1959  CA  LEU A 655      31.696  -8.763  16.103  1.00 36.09           C
ATOM   1961  CB  LEU A 655      30.302  -9.352  15.905  1.00 35.24           C
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ATOM   1964  CG   LEU A 655     29.159  -8.361  16.026  1.00 35.88           C
ATOM   1966  CD1  LEU A 655     27.882  -8.973  15.468  1.00 37.30           C
ATOM   1970  CD2  LEU A 655     29.000  -7.937  17.479  1.00 36.08           C
ATOM   1974  C    LEU A 655     32.749  -9.857  15.921  1.00 37.80           C
ATOM   1975  O    LEU A 655     32.686 -10.587  14.946  1.00 38.36           O
ATOM   1976  N    ASP A 656     33.700 -10.006  16.838  1.00 40.55           N
ATOM   1978  CA   ASP A 656     34.920 -10.783  16.530  1.00 42.89           C
ATOM   1980  CB   ASP A 656     36.142  -9.844  16.644  1.00 44.08           C
ATOM   1983  CG   ASP A 656     37.298 -10.225  15.710  1.00 48.10           C
ATOM   1984  OD1  ASP A 656     37.051 -10.684  14.565  1.00 52.44           O
ATOM   1985  OD2  ASP A 656     38.508 -10.062  16.043  1.00 54.53           O
ATOM   1986  C    ASP A 656     35.176 -12.045  17.381  1.00 43.63           C
ATOM   1987  O    ASP A 656     36.266 -12.637  17.299  1.00 43.86           O
ATOM   1988  N    HIS A 657     34.202 -12.481  18.176  1.00 44.15           N
ATOM   1990  CA   HIS A 657     34.493 -13.496  19.209  1.00 44.68           C
ATOM   1992  CB   HIS A 657     33.333 -13.615  20.208  1.00 44.74           C
ATOM   1995  CG   HIS A 657     33.742 -14.123  21.562  1.00 46.30           C
ATOM   1996  ND1  HIS A 657     32.944 -14.961  22.319  1.00 47.41           N
ATOM   1998  CE1  HIS A 657     33.557 -15.248  23.455  1.00 47.05           C
ATOM   2000  NE2  HIS A 657     34.726 -14.630  23.465  1.00 47.08           N
ATOM   2002  CD2  HIS A 657     34.866 -13.920  22.294  1.00 47.25           C
ATOM   2004  C    HIS A 657     34.902 -14.885  18.649  1.00 44.72           C
ATOM   2005  O    HIS A 657     34.141 -15.568  17.959  1.00 44.45           O
ATOM   2006  N    LEU A 672     45.942  -7.901  25.006  1.00 40.22           N
ATOM   2008  CA   LEU A 672     44.765  -7.106  25.373  1.00 39.72           C
ATOM   2010  CB   LEU A 672     44.699  -6.859  26.913  1.00 39.47           C
ATOM   2013  CG   LEU A 672     43.415  -7.305  27.648  1.00 41.50           C
ATOM   2015  CD1  LEU A 672     43.452  -6.944  29.155  1.00 42.61           C
ATOM   2019  CD2  LEU A 672     42.112  -6.783  27.000  1.00 42.23           C
ATOM   2023  C    LEU A 672     44.744  -5.790  24.557  1.00 38.16           C
ATOM   2024  O    LEU A 672     43.725  -5.460  23.975  1.00 39.19           O
ATOM   2025  N    ALA A 673     45.862  -5.067  24.492  1.00 37.22           N
ATOM   2027  CA   ALA A 673     45.987  -3.837  23.650  1.00 35.47           C
ATOM   2029  CB   ALA A 673     47.445  -3.430  23.512  1.00 35.51           C
ATOM   2033  C    ALA A 673     45.363  -3.964  22.279  1.00 34.63           C
ATOM   2034  O    ALA A 673     45.567  -4.963  21.609  1.00 34.37           O
ATOM   2035  N    GLN A 674     44.559  -2.973  21.877  1.00 33.43           N
ATOM   2037  CA   GLN A 674     43.895  -2.994  20.595  1.00 33.60           C
ATOM   2039  CB   GLN A 674     42.441  -2.497  20.707  1.00 34.24           C
ATOM   2042  CG   GLN A 674     41.513  -3.452  21.486  1.00 37.75           C
ATOM   2045  CD   GLN A 674     40.544  -4.242  20.641  1.00 38.80           C
ATOM   2046  OE1  GLN A 674     40.254  -3.910  19.474  1.00 44.44           O
ATOM   2047  NE2  GLN A 674     39.998  -5.288  21.241  1.00 43.56           N
ATOM   2050  C    GLN A 674     44.636  -2.149  19.546  1.00 32.40           C
ATOM   2051  O    GLN A 674     45.186  -1.073  19.851  1.00 31.00           O
ATOM   2052  N    LEU A 675     44.624  -2.667  18.328  1.00 30.90           N
ATOM   2054  CA   LEU A 675     45.211  -2.030  17.161  1.00 31.32           C
ATOM   2056  CB   LEU A 675     45.047  -2.949  15.927  1.00 30.92           C
ATOM   2059  CG   LEU A 675     45.868  -4.231  15.922  1.00 31.96           C
ATOM   2061  CD1  LEU A 675     45.312  -5.271  14.935  1.00 32.94           C
ATOM   2065  CD2  LEU A 675     47.374  -3.924  15.643  1.00 31.58           C
ATOM   2069  C    LEU A 675     44.462  -0.744  16.887  1.00 31.01           C
ATOM   2070  O    LEU A 675     43.268  -0.643  17.182  1.00 31.24           O
ATOM   2071  N    TYR A 676     45.154   0.234  16.326  1.00 30.42           N
ATOM   2073  CA   TYR A 676     44.500   1.431  15.814  1.00 30.40           C
ATOM   2075  CB   TYR A 676     45.470   2.333  15.026  1.00 30.49           C
ATOM   2078  CG   TYR A 676     45.057   3.757  15.120  1.00 29.31           C
ATOM   2079  CD1  TYR A 676     45.421   4.496  16.204  1.00 29.50           C
ATOM   2081  CE1  TYR A 676     45.044   5.813  16.339  1.00 31.10           C
ATOM   2083  CZ   TYR A 676     44.234   6.398  15.399  1.00 28.90           C
ATOM   2084  OH   TYR A 676     43.874   7.712  15.604  1.00 33.89           O
ATOM   2086  CE2  TYR A 676     43.817   5.686  14.301  1.00 30.81           C
ATOM   2088  CD2  TYR A 676     44.240   4.351  14.151  1.00 30.43           C
ATOM   2090  C    TYR A 676     43.347   1.066  14.920  1.00 31.11           C
ATOM   2091  O    TYR A 676     43.381   0.042  14.239  1.00 30.91           O
ATOM   2092  N    CYS A 677     42.325   1.903  14.918  1.00 32.33           N
ATOM   2094  CA   CYS A 677     41.246   1.853  13.902  1.00 34.86           C
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ATOM   2096  CB   CYS A 677   40.555   3.228  13.918  1.00 35.17      C
ATOM   2099  SG   CYS A 677   38.936   3.136  13.268  1.00 45.40      S
ATOM   2100  C    CYS A 677   41.718   1.627  12.430  1.00 33.82      C
ATOM   2101  O    CYS A 677   42.504   2.406  11.920  1.00 33.14      O
ATOM   2102  N    HIS A 678   41.188   0.596  11.765  1.00 34.07      N
ATOM   2104  CA   HIS A 678   41.471   0.227  10.363  1.00 33.24      C
ATOM   2106  CB   HIS A 678   41.112   1.358   9.401  1.00 34.61      C
ATOM   2109  CG   HIS A 678   39.763   1.949   9.632  1.00 35.54      C
ATOM   2110  ND1  HIS A 678   38.602   1.205   9.588  1.00 37.52      N
ATOM   2112  CE1  HIS A 678   37.569   1.999   9.805  1.00 34.44      C
ATOM   2114  NE2  HIS A 678   38.017   3.225   9.985  1.00 35.87      N
ATOM   2116  CD2  HIS A 678   39.386   3.220   9.885  1.00 35.92      C
ATOM   2118  C    HIS A 678   42.899  -0.232  10.041  1.00 33.03      C
ATOM   2119  O    HIS A 678   43.323  -0.198   8.892  1.00 33.36      O
ATOM   2120  N    SER A 679   43.630  -0.677  11.049  1.00 31.57      N
ATOM   2122  CA   SER A 679   44.947  -1.228  10.870  1.00 30.05      C
ATOM   2124  CB   SER A 679   45.432  -1.789  12.208  1.00 30.03      C
ATOM   2127  OG   SER A 679   46.508  -2.685  12.022  1.00 27.55      O
ATOM   2129  C    SER A 679   44.933  -2.374   9.854  1.00 30.06      C
ATOM   2130  O    SER A 679   44.007  -3.161   9.826  1.00 28.59      O
ATOM   2131  N    ILE A 680   46.001  -2.458   9.064  1.00 29.54      N
ATOM   2133  CA   ILE A 680   46.276  -3.552   8.133  1.00 30.65      C
ATOM   2135  CB   ILE A 680   47.564  -3.218   7.299  1.00 31.62      C
ATOM   2137  CG1  ILE A 680   47.396  -1.920   6.501  1.00 35.56      C
ATOM   2140  CD1  ILE A 680   48.607  -1.711   5.515  1.00 38.92      C
ATOM   2144  CG2  ILE A 680   47.878  -4.299   6.251  1.00 34.26      C
ATOM   2148  C    ILE A 680   46.435  -4.895   8.846  1.00 30.33      C
ATOM   2149  O    ILE A 680   46.355  -5.951   8.221  1.00 30.79      O
ATOM   2150  N    MET A 681   46.642  -4.857  10.158  1.00 29.68      N
ATOM   2152  CA   MET A 681   46.814  -6.057  10.931  1.00 30.17      C
ATOM   2154  CB   MET A 681   47.828  -5.794  12.070  1.00 29.84      C
ATOM   2157  CG   MET A 681   49.212  -5.461  11.539  1.00 31.17      C
ATOM   2160  SD   MET A 681   49.737  -6.803  10.421  1.00 33.29      S
ATOM   2161  CE   MET A 681   51.480  -6.883  10.639  1.00 34.00      C
ATOM   2165  C    MET A 681   45.498  -6.637  11.473  1.00 30.11      C
ATOM   2166  O    MET A 681   45.526  -7.716  11.991  1.00 29.99      O
ATOM   2167  N    GLU A 682   44.371  -5.941  11.318  1.00 30.83      N
ATOM   2169  CA   GLU A 682   43.083  -6.433  11.782  1.00 32.14      C
ATOM   2171  CB   GLU A 682   42.002  -5.357  11.801  1.00 32.53      C
ATOM   2174  CG   GLU A 682   42.241  -4.109  12.598  1.00 35.57      C
ATOM   2177  CD   GLU A 682   41.150  -3.074  12.378  1.00 38.08      C
ATOM   2178  OE1  GLU A 682   40.351  -3.244  11.428  1.00 38.05      O
ATOM   2179  OE2  GLU A 682   41.092  -2.089  13.152  1.00 38.67      O
ATOM   2180  C    GLU A 682   42.562  -7.526  10.868  1.00 32.35      C
ATOM   2181  O    GLU A 682   42.722  -7.455   9.649  1.00 32.87      O
ATOM   2182  N    HIS A 683   41.912  -8.515  11.468  1.00 32.50      N
ATOM   2184  CA   HIS A 683   41.286  -9.593  10.721  1.00 33.32      C
ATOM   2186  CB   HIS A 683   42.201 -10.824  10.637  1.00 32.66      C
ATOM   2189  CG   HIS A 683   43.316 -10.596   9.677  1.00 34.89      C
ATOM   2190  ND1  HIS A 683   43.082 -10.378   8.328  1.00 34.82      N
ATOM   2192  CE1  HIS A 683   44.226 -10.085   7.733  1.00 34.74      C
ATOM   2194  NE2  HIS A 683   45.183 -10.065   8.649  1.00 36.27      N
ATOM   2196  CD2  HIS A 683   44.631 -10.343   9.880  1.00 34.25      C
ATOM   2198  C    HIS A 683   39.924  -9.873  11.311  1.00 33.32      C
ATOM   2199  O    HIS A 683   39.811 -10.225  12.450  1.00 33.83      O
ATOM   2200  N    HIS A 684   38.899  -9.696  10.503  1.00 33.32      N
ATOM   2202  CA   HIS A 684   37.542  -9.800  10.968  1.00 33.77      C
ATOM   2204  CB   HIS A 684   36.660  -8.837  10.181  1.00 33.24      C
ATOM   2207  CG   HIS A 684   37.150  -7.446  10.227  1.00 35.04      C
ATOM   2208  ND1  HIS A 684   36.610  -6.499  11.072  1.00 37.86      N
ATOM   2210  CE1  HIS A 684   37.266  -5.365  10.921  1.00 34.96      C
ATOM   2212  NE2  HIS A 684   38.233  -5.553  10.043  1.00 33.74      N
ATOM   2214  CD2  HIS A 684   38.188  -6.850   9.604  1.00 32.56      C
ATOM   2216  C    HIS A 684   37.073 -11.221  10.762  1.00 33.63      C
ATOM   2217  O    HIS A 684   37.579 -11.936   9.898  1.00 34.39      O
ATOM   2218  N    HIS A 685   36.103 -11.618  11.563  1.00 33.78      N
ATOM   2220  CA   HIS A 685   35.509 -12.946  11.462  1.00 34.15      C
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ATOM   2222  CB   HIS A 685      35.955 -13.834  12.635  1.00 34.73           C
ATOM   2225  CG   HIS A 685      37.444 -13.941  12.772  1.00 37.59           C
ATOM   2226  ND1  HIS A 685      38.212 -14.721  11.933  1.00 42.39           N
ATOM   2228  CE1  HIS A 685      39.486 -14.589  12.255  1.00 41.15           C
ATOM   2230  NE2  HIS A 685      39.574 -13.749  13.268  1.00 40.09           N
ATOM   2232  CD2  HIS A 685      38.314 -13.318  13.603  1.00 39.95           C
ATOM   2234  C    HIS A 685      33.974 -12.866  11.360  1.00 32.94           C
ATOM   2235  O    HIS A 685      33.335 -11.946  11.820  1.00 32.00           O
ATOM   2236  N    PHE A 686      33.412 -13.870  10.741  1.00 33.17           N
ATOM   2238  CA   PHE A 686      32.008 -13.945  10.489  1.00 33.88           C
ATOM   2240  CB   PHE A 686      31.803 -14.491   9.088  1.00 35.01           C
ATOM   2243  CG   PHE A 686      32.525 -15.772   8.780  1.00 38.39           C
ATOM   2244  CD1  PHE A 686      32.167 -16.981   9.392  1.00 41.03           C
ATOM   2246  CE1  PHE A 686      32.803 -18.183   9.051  1.00 40.91           C
ATOM   2248  CZ   PHE A 686      33.804 -18.187   8.078  1.00 42.36           C
ATOM   2250  CE2  PHE A 686      34.157 -17.002   7.438  1.00 41.72           C
ATOM   2252  CD2  PHE A 686      33.515 -15.795   7.787  1.00 42.86           C
ATOM   2254  C    PHE A 686      31.292 -14.815  11.511  1.00 33.38           C
ATOM   2255  O    PHE A 686      30.065 -14.879  11.546  1.00 33.04           O
ATOM   2259  CG   PHE B 686      29.676 -15.568   8.280  1.00 50.77           C
ATOM   2260  CD1  PHE B 686      28.658 -14.648   8.564  1.00 51.19           C
ATOM   2262  CE1  PHE B 686      27.557 -14.841   7.742  1.00 51.49           C
ATOM   2264  CZ   PHE B 686      27.349 -16.162   7.300  1.00 51.14           C
ATOM   2266  CE2  PHE B 686      27.994 -17.198   8.051  1.00 51.13           C
ATOM   2268  CD2  PHE B 686      29.294 -16.908   8.397  1.00 50.89           C
ATOM   2272  N    ASP A 687      32.092 -15.582  12.599  1.00 31.50           N
ATOM   2274  CA   ASP A 687      31.473 -16.638  13.419  1.00 32.97           C
ATOM   2276  CB   ASP A 687      32.527 -17.298  14.298  1.00 33.51           C
ATOM   2279  CG   ASP A 687      33.782 -17.750  13.505  1.00 37.66           C
ATOM   2280  OD1  ASP A 687      34.365 -16.976  12.688  1.00 41.70           O
ATOM   2281  OD2  ASP A 687      34.275 -18.883  13.661  1.00 43.47           O
ATOM   2282  C    ASP A 687      30.352 -16.037  14.265  1.00 32.03           C
ATOM   2283  O    ASP A 687      29.286 -16.621  14.357  1.00 32.15           O
ATOM   2286  N    GLN A 688      30.565 -14.851  14.836  1.00 31.16           N
ATOM   2288  CA   GLN A 688      29.589 -14.264  15.750  1.00 31.25           C
ATOM   2290  CB   GLN A 688      30.225 -13.154  16.582  1.00 31.77           C
ATOM   2293  CG   GLN A 688      29.374 -12.759  17.799  1.00 34.88           C
ATOM   2296  CD   GLN A 688      30.119 -11.885  18.796  1.00 38.18           C
ATOM   2297  OE1  GLN A 688      31.181 -11.329  18.485  1.00 40.54           O
ATOM   2298  NE2  GLN A 688      29.559 -11.755  19.992  1.00 39.53           N
ATOM   2301  C    GLN A 688      28.353 -13.730  15.017  1.00 31.05           C
ATOM   2302  O    GLN A 688      27.220 -13.846  15.518  1.00 30.38           O
ATOM   2303  N    CYS A 689      28.555 -13.163  13.827  1.00 30.12           N
ATOM   2305  CA   CYS A 689      27.435 -12.763  12.967  1.00 30.44           C
ATOM   2307  CB   CYS A 689      27.949 -12.200  11.644  1.00 30.42           C
ATOM   2310  SG   CYS A 689      26.668 -11.902  10.426  1.00 30.66           S
ATOM   2311  C    CYS A 689      26.467 -13.923  12.677  1.00 30.97           C
ATOM   2312  O    CYS A 689      25.250 -13.779  12.825  1.00 31.28           O
ATOM   2313  N    LEU A 690      27.015 -15.064  12.276  1.00 31.79           N
ATOM   2315  CA   LEU A 690      26.236 -16.264  11.933  1.00 32.94           C
ATOM   2317  CB   LEU A 690      27.184 -17.331  11.368  1.00 33.46           C
ATOM   2320  CG   LEU A 690      26.757 -18.703  10.824  1.00 37.99           C
ATOM   2322  CD1  LEU A 690      26.703 -19.815  11.914  1.00 40.57           C
ATOM   2326  CD2  LEU A 690      25.420 -18.617  10.058  1.00 40.70           C
ATOM   2330  C    LEU A 690      25.538 -16.818  13.171  1.00 32.70           C
ATOM   2331  O    LEU A 690      24.375 -17.172  13.118  1.00 32.47           O
ATOM   2332  N    MET A 691      26.265 -16.907  14.284  1.00 33.18           N
ATOM   2334  CA   MET A 691      25.691 -17.403  15.534  1.00 34.16           C
ATOM   2336  CB   MET A 691      26.723 -17.390  16.651  1.00 35.01           C
ATOM   2339  CG   MET A 691      26.091 -17.561  18.050  1.00 40.17           C
ATOM   2342  SD   MET A 691      27.311 -17.341  19.371  1.00 50.88           S
ATOM   2343  CE   MET A 691      27.597 -15.483  19.419  1.00 47.95           C
ATOM   2347  C    MET A 691      24.449 -16.590  15.950  1.00 33.27           C
ATOM   2348  O    MET A 691      23.448 -17.160  16.358  1.00 32.30           O
ATOM   2349  N    ILE A 692      24.506 -15.266  15.816  1.00 32.39           N
ATOM   2351  CA   ILE A 692      23.360 -14.430  16.191  1.00 32.77           C
ATOM   2353  CB   ILE A 692      23.768 -12.937  16.271  1.00 32.53           C
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ATOM   2355  CG1 ILE A 692      24.784 -12.735  17.400  1.00 32.90           C
ATOM   2358  CD1 ILE A 692      25.376 -11.322  17.429  1.00 34.91           C
ATOM   2362  CG2 ILE A 692      22.547 -12.060  16.506  1.00 33.18           C
ATOM   2366  C   ILE A 692      22.173 -14.636  15.235  1.00 32.75           C
ATOM   2367  O   ILE A 692      21.019 -14.721  15.668  1.00 32.34           O
ATOM   2368  N   LEU A 693      22.476 -14.732  13.942  1.00 33.49           N
ATOM   2370  CA  LEU A 693      21.493 -14.980  12.882  1.00 33.88           C
ATOM   2372  CB  LEU A 693      22.191 -15.052  11.524  1.00 33.93           C
ATOM   2375  CG  LEU A 693      22.548 -13.756  10.805  1.00 35.20           C
ATOM   2377  CD1 LEU A 693      23.319 -14.084   9.540  1.00 35.75           C
ATOM   2381  CD2 LEU A 693      21.313 -12.992  10.457  1.00 37.26           C
ATOM   2385  C   LEU A 693      20.739 -16.291  13.056  1.00 34.18           C
ATOM   2386  O   LEU A 693      19.615 -16.427  12.571  1.00 34.35           O
ATOM   2387  N   ASN A 694      21.379 -17.266  13.690  1.00 34.61           N
ATOM   2389  CA  ASN A 694      20.787 -18.578  13.893  1.00 35.26           C
ATOM   2391  CB  ASN A 694      21.823 -19.662  13.585  1.00 36.11           C
ATOM   2394  CG  ASN A 694      22.160 -19.729  12.109  1.00 39.88           C
ATOM   2395  OD1 ASN A 694      21.322 -19.374  11.254  1.00 44.53           O
ATOM   2396  ND2 ASN A 694      23.390 -20.182  11.789  1.00 41.14           N
ATOM   2399  C   ASN A 694      20.231 -18.782  15.305  1.00 34.38           C
ATOM   2400  O   ASN A 694      19.838 -19.874  15.660  1.00 33.37           O
ATOM   2401  N   SER A 695      20.202 -17.715  16.089  1.00 33.65           N
ATOM   2403  CA  SER A 695      19.858 -17.780  17.499  1.00 33.45           C
ATOM   2405  CB  SER A 695      20.478 -16.562  18.191  1.00 33.53           C
ATOM   2408  OG  SER A 695      20.716 -16.834  19.539  1.00 36.56           O
ATOM   2410  C   SER A 695      18.347 -17.737  17.615  1.00 32.46           C
ATOM   2411  O   SER A 695      17.735 -16.981  16.874  1.00 31.34           O
ATOM   2412  N   PRO A 696      17.726 -18.528  18.506  1.00 32.72           N
ATOM   2413  CA  PRO A 696      16.253 -18.548  18.596  1.00 32.48           C
ATOM   2415  CB  PRO A 696      15.977 -19.410  19.837  1.00 32.89           C
ATOM   2418  CG  PRO A 696      17.170 -20.303  19.949  1.00 33.37           C
ATOM   2421  CD  PRO A 696      18.342 -19.465  19.468  1.00 32.62           C
ATOM   2424  C   PRO A 696      15.662 -17.153  18.773  1.00 32.13           C
ATOM   2425  O   PRO A 696      16.197 -16.391  19.560  1.00 32.27           O
ATOM   2426  N   GLY A 697      14.611 -16.825  18.023  1.00 31.88           N
ATOM   2428  CA  GLY A 697      13.997 -15.503  18.056  1.00 31.92           C
ATOM   2431  C   GLY A 697      14.697 -14.389  17.276  1.00 31.76           C
ATOM   2432  O   GLY A 697      14.146 -13.287  17.133  1.00 31.96           O
ATOM   2433  N   ASN A 698      15.890 -14.665  16.754  1.00 31.26           N
ATOM   2435  CA  ASN A 698      16.699 -13.663  16.060  1.00 31.31           C
ATOM   2437  CB  ASN A 698      18.093 -13.574  16.697  1.00 31.05           C
ATOM   2440  CG  ASN A 698      18.073 -13.015  18.099  1.00 30.32           C
ATOM   2441  OD1 ASN A 698      18.380 -11.869  18.298  1.00 30.29           O
ATOM   2442  ND2 ASN A 698      17.731 -13.836  19.078  1.00 32.05           N
ATOM   2445  C   ASN A 698      16.877 -13.999  14.580  1.00 31.57           C
ATOM   2446  O   ASN A 698      17.687 -13.374  13.900  1.00 31.67           O
ATOM   2447  N   GLN A 699      16.134 -14.990  14.084  1.00 31.49           N
ATOM   2449  CA  GLN A 699      16.391 -15.548  12.763  1.00 31.46           C
ATOM   2451  CB  GLN A 699      15.949 -17.022  12.691  1.00 32.07           C
ATOM   2454  CG  GLN A 699      16.781 -17.939  13.585  1.00 33.35           C
ATOM   2457  CD  GLN A 699      16.171 -19.315  13.780  1.00 36.39           C
ATOM   2458  OE1 GLN A 699      16.710 -20.309  13.298  1.00 37.88           O
ATOM   2459  NE2 GLN A 699      15.057 -19.378  14.500  1.00 38.71           N
ATOM   2462  C   GLN A 699      15.747 -14.709  11.674  1.00 31.10           C
ATOM   2463  O   GLN A 699      14.718 -15.079  11.104  1.00 30.82           O
ATOM   2464  N   ILE A 700      16.387 -13.592  11.346  1.00 30.47           N
ATOM   2466  CA  ILE A 700      15.834 -12.693  10.338  1.00 30.47           C
ATOM   2468  CB  ILE A 700      16.514 -11.300  10.394  1.00 30.43           C
ATOM   2470  CG1 ILE A 700      18.009 -11.385  10.130  1.00 32.01           C
ATOM   2473  CD1 ILE A 700      18.675 -10.020   9.846  1.00 32.43           C
ATOM   2477  CG2 ILE A 700      16.250 -10.653  11.752  1.00 29.96           C
ATOM   2481  C   ILE A 700      15.870 -13.260   8.908  1.00 30.26           C
ATOM   2482  O   ILE A 700      15.255 -12.701   8.037  1.00 29.34           O
ATOM   2483  N   LEU A 701      16.606 -14.346   8.679  1.00 30.75           N
ATOM   2485  CA  LEU A 701      16.675 -14.984   7.362  1.00 31.28           C
ATOM   2487  CB  LEU A 701      18.131 -15.364   7.008  1.00 30.89           C
ATOM   2490  CG  LEU A 701      19.172 -14.244   7.069  1.00 32.55           C
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ATOM   2492  CD1 LEU A 701      20.528 -14.746   6.632  1.00 33.04           C
ATOM   2496  CD2 LEU A 701      18.737 -13.067   6.230  1.00 32.79           C
ATOM   2500  C   LEU A 701      15.795 -16.219   7.268  1.00 31.50           C
ATOM   2501  O   LEU A 701      15.999 -17.024   6.371  1.00 31.76           O
ATOM   2502  N   SER A 702      14.805 -16.361   8.157  1.00 31.90           N
ATOM   2504  CA  SER A 702      13.963 -17.574   8.201  1.00 31.30           C
ATOM   2506  CB  SER A 702      13.101 -17.613   9.480  1.00 31.65           C
ATOM   2509  OG  SER A 702      12.202 -16.514   9.552  1.00 32.37           O
ATOM   2511  C   SER A 702      13.071 -17.704   6.966  1.00 30.98           C
ATOM   2512  O   SER A 702      12.822 -18.811   6.494  1.00 30.26           O
ATOM   2513  N   GLY A 703      12.652 -16.567   6.423  1.00 30.27           N
ATOM   2515  CA  GLY A 703      11.878 -16.506   5.196  1.00 30.37           C
ATOM   2518  C   GLY A 703      12.606 -16.916   3.907  1.00 30.71           C
ATOM   2519  O   GLY A 703      11.953 -17.174   2.893  1.00 30.10           O
ATOM   2520  N   LEU A 704      13.946 -16.971   3.936  1.00 30.77           N
ATOM   2522  CA  LEU A 704      14.726 -17.371   2.765  1.00 30.56           C
ATOM   2524  CB  LEU A 704      16.210 -16.996   2.909  1.00 30.10           C
ATOM   2527  CG  LEU A 704      16.723 -15.587   3.249  1.00 34.32           C
ATOM   2529  CD1 LEU A 704      18.193 -15.367   2.788  1.00 33.13           C
ATOM   2533  CD2 LEU A 704      15.867 -14.494   2.720  1.00 37.04           C
ATOM   2537  C   LEU A 704      14.635 -18.884   2.523  1.00 29.50           C
ATOM   2538  O   LEU A 704      14.684 -19.668   3.452  1.00 29.10           O
ATOM   2539  N   SER A 705      14.531 -19.281   1.261  1.00 28.82           N
ATOM   2541  CA  SER A 705      14.737 -20.665   0.851  1.00 28.42           C
ATOM   2543  CB  SER A 705      14.463 -20.819  -0.649  1.00 28.44           C
ATOM   2546  OG  SER A 705      15.424 -20.071  -1.386  1.00 28.13           O
ATOM   2548  C   SER A 705      16.175 -21.096   1.156  1.00 28.17           C
ATOM   2549  O   SER A 705      17.065 -20.251   1.338  1.00 27.96           O
ATOM   2550  N   ILE A 706      16.418 -22.400   1.213  1.00 28.12           N
ATOM   2552  CA  ILE A 706      17.751 -22.857   1.578  1.00 28.61           C
ATOM   2554  CB  ILE A 706      17.826 -24.376   1.797  1.00 29.31           C
ATOM   2556  CG1 ILE A 706      17.437 -25.161   0.549  1.00 29.59           C
ATOM   2559  CD1 ILE A 706      17.422 -26.641   0.794  1.00 33.51           C
ATOM   2563  CG2 ILE A 706      16.906 -24.781   2.941  1.00 30.87           C
ATOM   2567  C   ILE A 706      18.819 -22.337   0.614  1.00 28.18           C
ATOM   2568  O   ILE A 706      19.894 -21.953   1.060  1.00 27.65           O
ATOM   2569  N   GLU A 707      18.491 -22.243  -0.671  1.00 28.07           N
ATOM   2571  CA  GLU A 707      19.419 -21.740  -1.692  1.00 28.72           C
ATOM   2573  CB  GLU A 707      18.894 -22.020  -3.106  1.00 29.03           C
ATOM   2576  CG  GLU A 707      18.752 -23.486  -3.462  1.00 31.17           C
ATOM   2579  CD  GLU A 707      17.385 -24.097  -3.137  1.00 32.70           C
ATOM   2580  OE1 GLU A 707      16.497 -23.358  -2.611  1.00 31.18           O
ATOM   2581  OE2 GLU A 707      17.213 -25.329  -3.416  1.00 31.40           O
ATOM   2582  C   GLU A 707      19.704 -20.229  -1.517  1.00 28.17           C
ATOM   2583  O   GLU A 707      20.868 -19.790  -1.558  1.00 28.17           O
ATOM   2584  N   GLU A 708      18.657 -19.443  -1.313  1.00 27.81           N
ATOM   2586  CA  GLU A 708      18.801 -18.037  -0.910  1.00 28.54           C
ATOM   2588  CB  GLU A 708      17.451 -17.413  -0.577  1.00 28.31           C
ATOM   2591  CG  GLU A 708      16.613 -16.992  -1.766  1.00 30.10           C
ATOM   2594  CD  GLU A 708      15.335 -16.313  -1.319  1.00 29.90           C
ATOM   2595  OE1 GLU A 708      14.625 -16.911  -0.502  1.00 28.02           O
ATOM   2596  OE2 GLU A 708      15.061 -15.175  -1.751  1.00 32.16           O
ATOM   2597  C   GLU A 708      19.665 -17.873   0.348  1.00 28.37           C
ATOM   2598  O   GLU A 708      20.496 -16.971   0.436  1.00 27.41           O
ATOM   2599  N   TYR A 709      19.428 -18.739   1.327  1.00 28.74           N
ATOM   2601  CA  TYR A 709      20.109 -18.658   2.628  1.00 29.07           C
ATOM   2603  CB  TYR A 709      19.453 -19.633   3.636  1.00 29.15           C
ATOM   2606  CG  TYR A 709      20.124 -19.744   4.977  1.00 30.08           C
ATOM   2607  CD1 TYR A 709      19.868 -18.822   5.986  1.00 30.25           C
ATOM   2609  CE1 TYR A 709      20.487 -18.935   7.224  1.00 32.05           C
ATOM   2611  CZ  TYR A 709      21.352 -19.981   7.455  1.00 32.81           C
ATOM   2612  OH  TYR A 709      21.976 -20.115   8.665  1.00 36.64           O
ATOM   2614  CE2 TYR A 709      21.620 -20.907   6.470  1.00 31.98           C
ATOM   2616  CD2 TYR A 709      21.002 -20.790   5.248  1.00 30.61           C
ATOM   2618  C   TYR A 709      21.595 -18.948   2.419  1.00 28.39           C
ATOM   2619  O   TYR A 709      22.426 -18.153   2.792  1.00 27.85           O
ATOM   2620  N   LYS A 710      21.924 -20.058   1.773  1.00 29.18           N
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ATOM   2622  CA   LYS A 710    23.326 -20.390   1.544  1.00 30.08        C
ATOM   2624  CB   LYS A 710    23.482 -21.735   0.879  1.00 30.51        C
ATOM   2627  CG   LYS A 710    23.191 -22.928   1.801  1.00 32.10        C
ATOM   2630  CD   LYS A 710    23.256 -24.197   0.984  1.00 34.82        C
ATOM   2633  CE   LYS A 710    23.178 -25.471   1.823  1.00 36.19        C
ATOM   2636  NZ   LYS A 710    23.048 -26.659   0.962  1.00 36.81        N
ATOM   2640  C    LYS A 710    24.077 -19.287   0.758  1.00 30.38        C
ATOM   2641  O    LYS A 710    25.222 -18.983   1.078  1.00 30.06        O
ATOM   2642  N    THR A 711    23.419 -18.666  -0.222  1.00 29.86        N
ATOM   2644  CA   THR A 711    24.062 -17.629  -1.032  1.00 30.01        C
ATOM   2646  CB   THR A 711    23.217 -17.304  -2.280  1.00 30.66        C
ATOM   2648  OG1  THR A 711    23.216 -18.436  -3.175  1.00 30.28        O
ATOM   2650  CG2  THR A 711    23.857 -16.163  -3.073  1.00 30.73       .C
ATOM   2654  C    THR A 711    24.280 -16.369  -0.211  1.00 29.27        C
ATOM   2655  O    THR A 711    25.323 -15.703  -0.301  1.00 28.15        O
ATOM   2656  N    THR A 712    23.279 -16.032   0.594  1.00 29.05        N
ATOM   2658  CA   THR A 712    23.342 -14.843   1.409  1.00 28.20        C
ATOM   2660  CB   THR A 712    21.986 -14.624   2.128  1.00 28.55        C
ATOM   2662  OG1  THR A 712    20.947 -14.394   1.162  1.00 26.56        O
ATOM   2664  CG2  THR A 712    22.014 -13.347   2.972  1.00 28.61        C
ATOM   2668  C    THR A 712    24.486 -14.963   2.418  1.00 29.25        C
ATOM   2669  O    THR A 712    25.247 -14.001   2.655  1.00 28.91        O
ATOM   2670  N    LEU A 713    24.615 -16.130   3.030  1.00 28.84        N
ATOM   2672  CA   LEU A 713    25.679 -16.335   4.014  1.00 29.18        C
ATOM   2674  CB   LEU A 713    25.562 -17.704   4.709  1.00 29.57        C
ATOM   2677  CG   LEU A 713    24.344 -18.001   5.625  1.00 30.84        C
ATOM   2679  CD1  LEU A 713    24.694 -19.174   6.592  1.00 33.33        C
ATOM   2683  CD2  LEU A 713    23.816 -16.841   6.360  1.00 32.03        C
ATOM   2687  C    LEU A 713    27.054 -16.201   3.365  1.00 29.27        C
ATOM   2688  O    LEU A 713    27.971 -15.605   3.954  1.00 27.98        O
ATOM   2689  N    LYS A 714    27.203 -16.745   2.162  1.00 28.99        N
ATOM   2691  CA   LYS A 714    28.458 -16.634   1.431  1.00 29.77        C
ATOM   2693  CB   LYS A 714    28.397 -17.435   0.129  1.00 30.50        C
ATOM   2696  CG   LYS A 714    29.555 -17.190  -0.797  1.00 33.47        C
ATOM   2699  CD   LYS A 714    29.542 -18.049  -2.061  1.00 37.61        C
ATOM   2702  CE   LYS A 714    28.298 -17.795  -2.928  1.00 41.48        C
ATOM   2705  NZ   LYS A 714    27.560 -19.063  -3.353  1.00 42.85        N
ATOM   2709  C    LYS A 714    28.792 -15.172   1.154  1.00 29.88        C
ATOM   2710  O    LYS A 714    29.921 -14.753   1.379  1.00 30.34        O
ATOM   2711  N    ILE A 715    27.818 -14.368   0.724  1.00 29.41        N
ATOM   2713  CA   ILE A 715    28.087 -12.952   0.466  1.00 29.58        C
ATOM   2715  CB   ILE A 715    26.899 -12.252  -0.272  1.00 29.54        C
ATOM   2717  CG1  ILE A 715    26.630 -12.898  -1.640  1.00 30.27        C
ATOM   2720  CD1  ILE A 715    25.236 -12.628  -2.172  1.00 32.00        C
ATOM   2724  CG2  ILE A 715    27.202 -10.752  -0.452  1.00 30.82        C
ATOM   2728  C    ILE A 715    28.395 -12.199   1.773  1.00 30.15        C
ATOM   2729  O    ILE A 715    29.217 -11.283   1.780  1.00 29.32        O
ATOM   2730  N    ILE A 716    27.695 -12.540   2.865  1.00 30.66        N
ATOM   2732  CA   ILE A 716    27.948 -11.911   4.159  1.00 30.81        C
ATOM   2734  CB   ILE A 716    26.953 -12.400   5.284  1.00 30.93        C
ATOM   2736  CG1  ILE A 716    25.522 -11.939   5.007  1.00 29.96        C
ATOM   2739  CD1  ILE A 716    24.456 -12.555   5.941  1.00 28.36        C
ATOM   2743  CG2  ILE A 716    27.377 -11.825   6.666  1.00 32.78        C
ATOM   2747  C    ILE A 716    29.389 -12.147   4.618  1.00 30.65        C
ATOM   2748  O    ILE A 716    30.026 -11.204   5.071  1.00 31.73        O
ATOM   2749  N    LYS A 717    29.861 -13.389   4.530  1.00 31.34        N
ATOM   2751  CA   LYS A 717    31.224 -13.777   4.906  1.00 32.36        C
ATOM   2753  CB   LYS A 717    31.507 -15.261   4.657  1.00 32.51        C
ATOM   2756  CG   LYS A 717    30.760 -16.269   5.531  1.00 35.68        C
ATOM   2759  CD   LYS A 717    31.032 -17.766   5.106  1.00 38.43        C
ATOM   2762  CE   LYS A 717    30.092 -18.775   5.818  1.00 39.94        C
ATOM   2765  NZ   LYS A 717    29.895 -20.095   5.141  1.00 39.44        N
ATOM   2769  C    LYS A 717    32.239 -12.986   4.095  1.00 32.41        C
ATOM   2770  O    LYS A 717    33.140 -12.387   4.670  1.00 31.82        O
ATOM   2771  N    GLN A 718    32.091 -13.001   2.769  1.00 31.49        N
ATOM   2773  CA   GLN A 718    32.952 -12.216   1.880  1.00 32.27        C
ATOM   2775  CB   GLN A 718    32.541 -12.381   0.410  1.00 32.06        C
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ATOM	2778	CG	GLN	A	718	32.935	-13.729	-0.165	1.00	33.82	C
ATOM	2781	CD	GLN	A	718	32.343	-14.005	-1.544	1.00	36.85	C
ATOM	2782	OE1	GLN	A	718	31.380	-13.347	-1.972	1.00	39.56	O
ATOM	2783	NE2	GLN	A	718	32.923	-14.983	-2.246	1.00	37.92	N
ATOM	2786	C	GLN	A	718	32.958	-10.738	2.257	1.00	31.81	C
ATOM	2787	O	GLN	A	718	34.004	-10.123	2.318	1.00	31.71	O
ATOM	2788	N	ALA	A	719	31.792	-10.182	2.549	1.00	31.68	N
ATOM	2790	CA	ALA	A	719	31.683	-8.762	2.853	1.00	31.63	C
ATOM	2792	CB	ALA	A	719	30.234	-8.325	2.816	1.00	32.00	C
ATOM	2796	C	ALA	A	719	32.312	-8.380	4.189	1.00	32.00	C
ATOM	2797	O	ALA	A	719	32.860	-7.270	4.358	1.00	31.34	O
ATOM	2798	N	ILE	A	720	32.228	-9.284	5.158	1.00	32.32	N
ATOM	2800	CA	ILE	A	720	32.846	-9.021	6.455	1.00	31.42	C
ATOM	2802	CB	ILE	A	720	32.323	-9.983	7.534	1.00	30.69	C
ATOM	2804	CG1	ILE	A	720	30.901	-9.615	7.919	1.00	27.83	C
ATOM	2807	CD1	ILE	A	720	30.313	-10.502	8.957	1.00	27.29	C
ATOM	2811	CG2	ILE	A	720	33.235	-9.947	8.747	1.00	31.46	C
ATOM	2815	C	ILE	A	720	34.354	-9.146	6.316	1.00	31.68	C
ATOM	2816	O	ILE	A	720	35.095	-8.321	6.851	1.00	31.13	O
ATOM	2817	N	LEU	A	721	34.803	-10.186	5.612	1.00	32.18	N
ATOM	2819	CA	LEU	A	721	36.223	-10.374	5.345	1.00	32.91	C
ATOM	2821	CB	LEU	A	721	36.529	-11.711	4.675	1.00	32.99	C
ATOM	2824	CG	LEU	A	721	36.297	-12.971	5.525	1.00	37.70	C
ATOM	2826	CD1	LEU	A	721	36.514	-14.245	4.683	1.00	38.76	C
ATOM	2830	CD2	LEU	A	721	37.162	-13.046	6.745	1.00	40.11	C
ATOM	2834	C	LEU	A	721	36.794	-9.216	4.524	1.00	32.85	C
ATOM	2835	O	LEU	A	721	37.952	-8.836	4.754	1.00	33.57	O
ATOM	2836	N	ALA	A	722	35.986	-8.634	3.630	1.00	31.29	N
ATOM	2838	CA	ALA	A	722	36.363	-7.441	2.873	1.00	31.32	C
ATOM	2840	CB	ALA	A	722	35.201	-6.986	1.947	1.00	31.27	C
ATOM	2844	C	ALA	A	722	36.773	-6.233	3.752	1.00	30.62	C
ATOM	2845	O	ALA	A	722	37.454	-5.358	3.264	1.00	29.59	O
ATOM	2846	N	THR	A	723	36.306	-6.143	5.000	1.00	30.85	N
ATOM	2848	CA	THR	A	723	36.697	-5.038	5.883	1.00	31.23	C
ATOM	2850	CB	THR	A	723	35.712	-4.775	7.046	1.00	30.40	C
ATOM	2852	OG1	THR	A	723	35.606	-5.914	7.911	1.00	32.47	O
ATOM	2854	CG2	THR	A	723	34.344	-4.558	6.528	1.00	32.10	C
ATOM	2858	C	THR	A	723	38.086	-5.173	6.447	1.00	30.74	C
ATOM	2859	O	THR	A	723	38.563	-4.269	7.109	1.00	30.67	O
ATOM	2860	N	ASP	A	724	38.748	-6.280	6.178	1.00	31.33	N
ATOM	2862	CA	ASP	A	724	40.189	-6.380	6.428	1.00	31.24	C
ATOM	2864	CB	ASP	A	724	40.733	-7.764	6.088	1.00	30.66	C
ATOM	2867	CG	ASP	A	724	40.290	-8.837	7.030	1.00	29.75	C
ATOM	2868	OD1	ASP	A	724	39.440	-8.600	7.884	1.00	29.76	O
ATOM	2869	OD2	ASP	A	724	40.762	-9.987	6.987	1.00	30.86	O
ATOM	2870	C	ASP	A	724	40.851	-5.405	5.454	1.00	32.00	C
ATOM	2871	O	ASP	A	724	40.796	-5.626	4.228	1.00	31.56	O
ATOM	2872	N	LEU	A	725	41.451	-4.324	5.949	1.00	30.56	N
ATOM	2874	CA	LEU	A	725	42.126	-3.402	5.030	1.00	30.51	C
ATOM	2876	CB	LEU	A	725	42.814	-2.254	5.771	1.00	31.33	C
ATOM	2879	CG	LEU	A	725	42.583	-0.759	5.485	1.00	34.63	C
ATOM	2881	CD1	LEU	A	725	43.914	-0.011	5.423	1.00	36.43	C
ATOM	2885	CD2	LEU	A	725	41.630	-0.346	4.388	1.00	32.58	C
ATOM	2889	C	LEU	A	725	43.204	-4.053	4.203	1.00	29.44	C
ATOM	2890	O	LEU	A	725	43.438	-3.601	3.108	1.00	29.73	O
ATOM	2891	N	ALA	A	726	43.911	-5.052	4.731	1.00	28.27	N
ATOM	2893	CA	ALA	A	726	44.933	-5.746	3.953	1.00	29.03	C
ATOM	2895	CB	ALA	A	726	45.675	-6.803	4.775	1.00	28.55	C
ATOM	2899	C	ALA	A	726	44.357	-6.370	2.667	1.00	29.70	C
ATOM	2900	O	ALA	A	726	45.053	-6.475	1.677	1.00	28.98	O
ATOM	2901	N	LEU	A	727	43.099	-6.779	2.706	1.00	30.51	N
ATOM	2903	CA	LEU	A	727	42.458	-7.400	1.552	1.00	32.05	C
ATOM	2905	CB	LEU	A	727	41.218	-8.177	1.982	1.00	32.66	C
ATOM	2908	CG	LEU	A	727	41.369	-9.660	2.296	1.00	37.39	C
ATOM	2910	CD1	LEU	A	727	39.942	-10.265	2.429	1.00	39.88	C
ATOM	2914	CD2	LEU	A	727	42.170	-10.453	1.259	1.00	38.45	C
ATOM	2918	C	LEU	A	727	42.044	-6.313	0.546	1.00	30.69	C

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ATOM   2919  O    LEU A 727      42.123  -6.514  -0.637  1.00 29.90           O
ATOM   2920  N    TYR A 728      41.626  -5.166   1.060  1.00 30.15           N
ATOM   2922  CA   TYR A 728      41.334  -4.004   0.242  1.00 29.75           C
ATOM   2924  CB   TYR A 728      40.822  -2.832   1.087  1.00 30.44           C
ATOM   2927  CG   TYR A 728      40.951  -1.498   0.372  1.00 31.40           C
ATOM   2928  CD1  TYR A 728      40.157  -1.192  -0.715  1.00 32.68           C
ATOM   2930  CE1  TYR A 728      40.296   0.029  -1.386  1.00 33.60           C
ATOM   2932  CZ   TYR A 728      41.232   0.939  -0.956  1.00 32.26           C
ATOM   2933  OH   TYR A 728      41.386   2.148  -1.597  1.00 33.27           O
ATOM   2935  CE2  TYR A 728      42.044   0.640   0.111  1.00 32.66           C
ATOM   2937  CD2  TYR A 728      41.888  -0.555   0.782  1.00 31.84           C
ATOM   2939  C    TYR A 728      42.582  -3.607  -0.521  1.00 29.20           C
ATOM   2940  O    TYR A 728      42.541  -3.458  -1.739  1.00 28.20           O
ATOM   2941  N    ILE A 729      43.713  -3.516   0.173  1.00 27.85           N
ATOM   2943  CA   ILE A 729      44.937  -3.036  -0.461  1.00 27.07           C
ATOM   2945  CB   ILE A 729      46.028  -2.741   0.603  1.00 26.18           C
ATOM   2947  CG1  ILE A 729      45.600  -1.552   1.458  1.00 27.32           C
ATOM   2950  CD1  ILE A 729      46.362  -1.426   2.761  1.00 29.88           C
ATOM   2954  CG2  ILE A 729      47.358  -2.423  -0.087  1.00 25.82           C
ATOM   2958  C    ILE A 729      45.428  -4.032  -1.491  1.00 26.41           C
ATOM   2959  O    ILE A 729      45.980  -3.646  -2.520  1.00 25.30           O
ATOM   2960  N    LYS A 730      45.234  -5.309  -1.183  1.00 26.14           N
ATOM   2962  CA   LYS A 730      45.673  -6.381  -2.046  1.00 27.40           C
ATOM   2964  CB   LYS A 730      45.487  -7.734  -1.346  1.00 26.89           C
ATOM   2967  CG   LYS A 730      45.929  -8.938  -2.203  1.00 29.59           C
ATOM   2970  CD   LYS A 730      45.470 -10.258  -1.580  1.00 33.15           C
ATOM   2973  CE   LYS A 730      45.912 -11.470  -2.371  1.00 34.65           C
ATOM   2976  NZ   LYS A 730      45.545 -12.673  -1.583  1.00 38.38           N
ATOM   2980  C    LYS A 730      44.904  -6.360  -3.389  1.00 27.18           C
ATOM   2981  O    LYS A 730      45.489  -6.603  -4.464  1.00 26.31           O
ATOM   2982  N    ARG A 731      43.606  -6.106  -3.316  1.00 27.73           N
ATOM   2984  CA   ARG A 731      42.702  -6.333  -4.469  1.00 29.48           C
ATOM   2986  CB   ARG A 731      41.423  -7.017  -3.999  1.00 29.63           C
ATOM   2989  CG   ARG A 731      41.621  -8.516  -3.662  1.00 34.45           C
ATOM   2992  CD   ARG A 731      40.333  -9.179  -3.157  1.00 39.61           C
ATOM   2995  NE   ARG A 731      39.558  -9.710  -4.292  1.00 45.03           N
ATOM   2997  CZ   ARG A 731      38.213  -9.788  -4.350  1.00 45.41           C
ATOM   2998  NH1  ARG A 731      37.438  -9.436  -3.329  1.00 45.49           N
ATOM   3001  NH2  ARG A 731      37.644 -10.258  -5.447  1.00 47.21           N
ATOM   3004  C    ARG A 731      42.354  -5.067  -5.266  1.00 29.31           C
ATOM   3005  O    ARG A 731      41.908  -5.152  -6.416  1.00 28.60           O
ATOM   3006  N    ARG A 732      42.573  -3.902  -4.661  1.00 29.07           N
ATOM   3008  CA   ARG A 732      42.095  -2.660  -5.242  1.00 29.47           C
ATOM   3010  CB   ARG A 732      42.269  -1.481  -4.300  1.00 29.23           C
ATOM   3013  CG   ARG A 732      43.695  -1.030  -4.066  1.00 29.95           C
ATOM   3016  CD   ARG A 732      43.791   0.013  -2.935  1.00 28.49           C
ATOM   3019  NE   ARG A 732      45.155   0.519  -2.808  1.00 31.38           N
ATOM   3021  CZ   ARG A 732      45.491   1.713  -2.325  1.00 28.56           C
ATOM   3022  NH1  ARG A 732      44.585   2.561  -1.890  1.00 29.84           N
ATOM   3025  NH2  ARG A 732      46.751   2.056  -2.301  1.00 30.13           N
ATOM   3028  C    ARG A 732      42.718  -2.324  -6.580  1.00 30.07           C
ATOM   3029  O    ARG A 732      42.039  -1.750  -7.401  1.00 30.71           O
ATOM   3030  N    GLY A 733      43.987  -2.663  -6.797  1.00 29.98           N
ATOM   3032  CA   GLY A 733      44.666  -2.401  -8.063  1.00 30.89           C
ATOM   3035  C    GLY A 733      43.915  -2.924  -9.281  1.00 32.03           C
ATOM   3036  O    GLY A 733      43.810  -2.237 -10.323  1.00 31.34           O
ATOM   3037  N    GLU A 734      43.384  -4.141  -9.150  1.00 33.29           N
ATOM   3039  CA   GLU A 734      42.573  -4.741 -10.209  1.00 34.28           C
ATOM   3041  CB   GLU A 734      42.197  -6.194  -9.861  1.00 34.83           C
ATOM   3044  CG   GLU A 734      41.219  -6.849 -10.858  1.00 36.43           C
ATOM   3047  CD   GLU A 734      40.859  -8.284 -10.513  1.00 37.93           C
ATOM   3048  OE1  GLU A 734      40.857  -8.644  -9.318  1.00 38.78           O
ATOM   3049  OE2  GLU A 734      40.574  -9.058 -11.458  1.00 41.92           O
ATOM   3050  C    GLU A 734      41.303  -3.904 -10.450  1.00 33.90           C
ATOM   3051  O    GLU A 734      40.929  -3.652 -11.592  1.00 34.11           O
ATOM   3052  N    PHE A 735      40.635  -3.489  -9.374  1.00 34.01           N
ATOM   3054  CA   PHE A 735      39.474  -2.601  -9.476  1.00 33.89           C
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ATOM   3056   CB    PHE A 735      38.942   -2.296   -8.078   1.00 34.08         C
ATOM   3059   CG    PHE A 735      37.713   -1.439   -8.042   1.00 36.84         C
ATOM   3060   CD1   PHE A 735      36.594   -1.745   -8.815   1.00 39.84         C
ATOM   3062   CE1   PHE A 735      35.452   -0.962   -8.756   1.00 40.64         C
ATOM   3064   CZ    PHE A 735      35.407    0.133   -7.910   1.00 41.90         C
ATOM   3066   CE2   PHE A 735      36.512    0.437   -7.105   1.00 40.61         C
ATOM   3068   CD2   PHE A 735      37.650   -0.347   -7.180   1.00 39.10         C
ATOM   3070   C     PHE A 735      39.814   -1.302  -10.191   1.00 33.50         C
ATOM   3071   O     PHE A 735      39.151   -0.934  -11.180   1.00 33.37         O
ATOM   3072   N     PHE A 736      40.844   -0.616   -9.704   1.00 33.05         N
ATOM   3074   CA    PHE A 736      41.217    0.699  -10.206   1.00 34.06         C
ATOM   3076   CB    PHE A 736      42.366    1.295   -9.392   1.00 34.30         C
ATOM   3079   CG    PHE A 736      42.024    1.593   -7.941   1.00 35.16         C
ATOM   3080   CD1   PHE A 736      43.043    1.852   -7.027   1.00 35.56         C
ATOM   3082   CE1   PHE A 736      42.751    2.126   -5.726   1.00 37.00         C
ATOM   3084   CZ    PHE A 736      41.436    2.123   -5.300   1.00 38.26         C
ATOM   3086   CE2   PHE A 736      40.421    1.854   -6.195   1.00 36.37         C
ATOM   3088   CD2   PHE A 736      40.714    1.598   -7.490   1.00 34.99         C
ATOM   3090   C     PHE A 736      41.612    0.665  -11.689   1.00 35.47         C
ATOM   3091   O     PHE A 736      41.337    1.620  -12.416   1.00 35.44         O
ATOM   3092   N     GLU A 737      42.224   -0.447  -12.116   1.00 35.60         N
ATOM   3094   CA    GLU A 737      42.700   -0.632  -13.476   1.00 36.57         C
ATOM   3096   CB    GLU A 737      43.742   -1.765  -13.529   1.00 36.39         C
ATOM   3099   CG    GLU A 737      44.284   -2.096  -14.912   1.00 38.88         C
ATOM   3102   CD    GLU A 737      45.512   -1.281  -15.295   1.00 41.05         C
ATOM   3103   OE1   GLU A 737      45.690   -0.154  -14.778   1.00 42.96         O
ATOM   3104   OE2   GLU A 737      46.300   -1.772  -16.131   1.00 42.28         O
ATOM   3105   C     GLU A 737      41.535   -0.931  -14.421   1.00 36.83         C
ATOM   3106   O     GLU A 737      41.483   -0.395  -15.521   1.00 35.58         O
ATOM   3107   N     LEU A 738      40.615   -1.790  -13.994   1.00 37.36         N
ATOM   3109   CA    LEU A 738      39.409   -2.021  -14.773   1.00 37.62         C
ATOM   3111   CB    LEU A 738      38.474   -3.008  -14.064   1.00 37.25         C
ATOM   3114   CG    LEU A 738      38.872   -4.481  -13.963   1.00 36.35         C
ATOM   3116   CD1   LEU A 738      37.998   -5.192  -12.939   1.00 36.30         C
ATOM   3120   CD2   LEU A 738      38.785   -5.189  -15.300   1.00 37.05         C
ATOM   3124   C     LEU A 738      38.685   -0.688  -15.054   1.00 38.70         C
ATOM   3125   O     LEU A 738      38.291   -0.438  -16.174   1.00 39.15         O
ATOM   3126   N     ILE A 739      38.523    0.172  -14.050   1.00 40.41         N
ATOM   3128   CA    ILE A 739      37.810    1.447  -14.227   1.00 41.58         C
ATOM   3130   CB    ILE A 739      37.419    2.036  -12.858   1.00 41.98         C
ATOM   3132   CG1   ILE A 739      36.243    1.242  -12.282   1.00 41.05         C
ATOM   3135   CD1   ILE A 739      36.094    1.368  -10.859   1.00 41.49         C
ATOM   3139   CG2   ILE A 739      37.024    3.520  -12.973   1.00 42.55         C
ATOM   3143   C     ILE A 739      38.594    2.472  -15.070   1.00 43.09         C
ATOM   3144   O     ILE A 739      38.010    3.155  -15.921   1.00 43.46         O
ATOM   3145   N     ARG A 740      39.902    2.565  -14.828   1.00 43.89         N
ATOM   3147   CA    ARG A 740      40.805    3.523  -15.480   1.00 44.29         C
ATOM   3149   CB    ARG A 740      42.229    3.264  -14.978   1.00 44.62         C
ATOM   3152   CG    ARG A 740      43.352    4.163  -15.486   1.00 45.76         C
ATOM   3155   CD    ARG A 740      44.704    3.845  -14.808   1.00 46.92         C
ATOM   3158   NE    ARG A 740      44.528    3.637  -13.357   1.00 49.06         N
ATOM   3160   CZ    ARG A 740      45.175    2.735  -12.593   1.00 47.38         C
ATOM   3161   NH1   ARG A 740      46.087    1.913  -13.098   1.00 47.75         N
ATOM   3164   NH2   ARG A 740      44.900    2.671  -11.306   1.00 46.41         N
ATOM   3167   C     ARG A 740      40.731    3.329  -16.979   1.00 44.59         C
ATOM   3168   O     ARG A 740      40.689    4.294  -17.759   1.00 44.64         O
ATOM   3169   N     LYS A 741      40.714    2.060  -17.369   1.00 44.87         N
ATOM   3171   CA    LYS A 741      40.325    1.653  -18.706   1.00 45.02         C
ATOM   3173   CB    LYS A 741      40.644    0.170  -18.883   1.00 45.13         C
ATOM   3176   CG    LYS A 741      42.115   -0.177  -18.715   1.00 44.76         C
ATOM   3179   CD    LYS A 741      42.381   -1.583  -19.222   1.00 44.18         C
ATOM   3182   CE    LYS A 741      43.806   -2.043  -18.913   1.00 44.31         C
ATOM   3185   NZ    LYS A 741      44.462   -2.620  -20.128   1.00 43.46         N
ATOM   3189   C     LYS A 741      38.816    1.933  -18.812   1.00 45.27         C
ATOM   3190   O     LYS A 741      38.364    3.001  -18.383   1.00 45.85         O
ATOM   3191   N     ASN A 742      38.028    1.010  -19.358   1.00 44.90         N
ATOM   3193   CA    ASN A 742      36.571    1.065  -19.175   1.00 44.48         C
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ATOM   3195   CB    ASN A 742      35.915    2.030  -20.184   1.00  44.33           C
ATOM   3198   CG    ASN A 742      35.926    3.472  -19.702   1.00  44.37           C
ATOM   3199   OD1   ASN A 742      35.169    3.857  -18.800   1.00  43.50           O
ATOM   3200   ND2   ASN A 742      36.807    4.274  -20.284   1.00  44.38           N
ATOM   3203   C     ASN A 742      36.016   -0.336  -19.333   1.00  44.23           C
ATOM   3204   O     ASN A 742      35.114   -0.581  -20.155   1.00  44.09           O
ATOM   3205   N     GLN A 743      36.598   -1.257  -18.572   1.00  43.32           N
ATOM   3207   CA    GLN A 743      36.328   -2.679  -18.738   1.00  43.18           C
ATOM   3209   CB    GLN A 743      37.643   -3.457  -18.878   1.00  42.99           C
ATOM   3212   CG    GLN A 743      38.443   -3.088  -20.134   1.00  44.00           C
ATOM   3215   CD    GLN A 743      39.778   -3.818  -20.243   1.00  44.62           C
ATOM   3216   OE1   GLN A 743      40.174   -4.554  -19.332   1.00  45.85           O
ATOM   3217   NE2   GLN A 743      40.478   -3.604  -21.351   1.00  44.62           N
ATOM   3220   C     GLN A 743      35.517   -3.216  -17.584   1.00  42.81           C
ATOM   3221   O     GLN A 743      35.214   -4.411  -17.539   1.00  42.59           O
ATOM   3222   N     PHE A 744      35.150   -2.328  -16.657   1.00  43.16           N
ATOM   3224   CA    PHE A 744      34.518   -2.740  -15.422   1.00  42.76           C
ATOM   3226   CB    PHE A 744      34.455   -1.587  -14.429   1.00  43.01           C
ATOM   3229   CG    PHE A 744      33.629   -1.899  -13.220   1.00  43.65           C
ATOM   3230   CD1   PHE A 744      33.922   -3.012  -12.438   1.00  43.33           C
ATOM   3232   CE1   PHE A 744      33.161   -3.319  -11.324   1.00  43.62           C
ATOM   3234   CZ    PHE A 744      32.083   -2.523  -10.987   1.00  44.49           C
ATOM   3236   CE2   PHE A 744      31.767   -1.417  -11.772   1.00  45.02           C
ATOM   3238   CD2   PHE A 744      32.535   -1.115  -12.886   1.00  44.45           C
ATOM   3240   C     PHE A 744      33.123   -3.218  -15.731   1.00  42.74           C
ATOM   3241   O     PHE A 744      32.390   -2.523  -16.412   1.00  42.85           O
ATOM   3242   N     ASN A 745      32.757   -4.384  -15.202   1.00  42.84           N
ATOM   3244   CA    ASN A 745      31.540   -5.082  -15.592   1.00  43.14           C
ATOM   3246   CB    ASN A 745      31.825   -5.887  -16.871   1.00  43.72           C
ATOM   3249   CG    ASN A 745      30.967   -5.444  -18.051   1.00  44.57           C
ATOM   3250   OD1   ASN A 745      29.836   -4.996  -17.874   1.00  47.27           O
ATOM   3251   ND2   ASN A 745      31.506   -5.571  -19.265   1.00  46.68           N
ATOM   3254   C     ASN A 745      30.936   -5.994  -14.498   1.00  43.33           C
ATOM   3255   O     ASN A 745      31.463   -7.082  -14.212   1.00  43.25           O
ATOM   3256   N     LEU A 746      29.817   -5.538  -13.914   1.00  43.48           N
ATOM   3258   CA    LEU A 746      29.080   -6.217  -12.834   1.00  43.30           C
ATOM   3260   CB    LEU A 746      27.926   -5.322  -12.379   1.00  43.40           C
ATOM   3263   CG    LEU A 746      28.272   -4.048  -11.577   1.00  43.36           C
ATOM   3265   CD1   LEU A 746      28.023   -2.727  -12.339   1.00  43.92           C
ATOM   3269   CD2   LEU A 746      27.460   -4.041  -10.334   1.00  43.29           C
ATOM   3273   C     LEU A 746      28.512   -7.603  -13.176   1.00  43.97           C
ATOM   3274   O     LEU A 746      28.046   -8.334  -12.298   1.00  44.14           O
ATOM   3275   N     GLU A 747      28.510   -7.927  -14.462   1.00  44.34           N
ATOM   3277   CA    GLU A 747      28.263   -9.275  -14.966   1.00  44.73           C
ATOM   3279   CB    GLU A 747      28.358   -9.226  -16.491   1.00  44.60           C
ATOM   3282   CG    GLU A 747      29.512   -8.370  -16.999   1.00  44.39           C
ATOM   3285   CD    GLU A 747      30.170   -8.919  -18.260   1.00  45.57           C
ATOM   3286   OE1   GLU A 747      29.449   -9.389  -19.204   1.00  42.97           O
ATOM   3287   OE2   GLU A 747      31.427   -8.864  -18.300   1.00  45.91           O
ATOM   3288   C     GLU A 747      29.154  -10.451  -14.464   1.00  45.39           C
ATOM   3289   O     GLU A 747      28.641  -11.570  -14.274   1.00  45.81           O
ATOM   3290   N     ASP A 748      30.467  -10.252  -14.308   1.00  45.36           N
ATOM   3292   CA    ASP A 748      31.318  -11.384  -13.935   1.00  45.44           C
ATOM   3294   CB    ASP A 748      32.860  -11.184  -14.061   1.00  45.79           C
ATOM   3297   CG    ASP A 748      33.291  -10.175  -15.111   1.00  47.52           C
ATOM   3298   OD1   ASP A 748      34.526  -10.021  -15.246   1.00  50.96           O
ATOM   3299   OD2   ASP A 748      32.531   -9.496  -15.839   1.00  49.90           O
ATOM   3300   C     ASP A 748      31.042  -11.589  -12.460   1.00  44.95           C
ATOM   3301   O     ASP A 748      31.201  -10.649  -11.686   1.00  44.92           O
ATOM   3302   N     PRO A 749      30.710  -12.814  -12.057   1.00  44.38           N
ATOM   3303   CA    PRO A 749      30.700  -13.157  -10.633   1.00  44.02           C
ATOM   3305   CB    PRO A 749      30.837  -14.686  -10.632   1.00  44.29           C
ATOM   3308   CG    PRO A 749      30.281  -15.142  -11.954   1.00  43.95           C
ATOM   3311   CD    PRO A 749      30.386  -13.975  -12.909   1.00  44.40           C
ATOM   3314   C     PRO A 749      31.897  -12.524   -9.906   1.00  43.81           C
ATOM   3315   O     PRO À 749      31.727  -12.026   -8.792   1.00  43.90           O
ATOM   3316   N     HIS A 750      33.068  -12.525  -10.545   1.00  43.08           N
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ATOM   3318  CA   HIS A 750      34.294 -12.034  -9.926  1.00 42.92          C
ATOM   3320  CB   HIS A 750      35.543 -12.469 -10.734  1.00 43.34          C
ATOM   3323  CG   HIS A 750      36.816 -12.062 -10.070  1.00 45.39          C
ATOM   3324  ND1  HIS A 750      37.534 -10.951 -10.458  1.00 46.61          N
ATOM   3326  CE1  HIS A 750      38.566 -10.800  -9.649  1.00 47.97          C
ATOM   3328  NE2  HIS A 750      38.526 -11.750  -8.732  1.00 48.77          N
ATOM   3330  CD2  HIS A 750      37.432 -12.544  -8.964  1.00 48.45          C
ATOM   3332  C    HIS A 750      34.311 -10.509  -9.732  1.00 41.70          C
ATOM   3333  O    HIS A 750      34.591 -10.027  -8.646  1.00 41.56          O
ATOM   3334  N    GLN A 751      34.038  -9.758 -10.791  1.00 40.61          N
ATOM   3336  CA   GLN A 751      34.014  -8.301 -10.705  1.00 39.95          C
ATOM   3338  CB   GLN A 751      33.881  -7.693 -12.098  1.00 39.30          C
ATOM   3341  CG   GLN A 751      35.122  -7.943 -12.951  1.00 38.86          C
ATOM   3344  CD   GLN A 751      35.153  -7.152 -14.235  1.00 36.74          C
ATOM   3345  OE1  GLN A 751      34.985  -5.946 -14.232  1.00 38.17          O
ATOM   3346  NE2  GLN A 751      35.388  -7.826 -15.325  1.00 35.87          N
ATOM   3349  C    GLN A 751      32.914  -7.793  -9.762  1.00 40.04          C
ATOM   3350  O    GLN A 751      33.059  -6.721  -9.140  1.00 40.25          O
ATOM   3351  N    LYS A 752      31.842  -8.581  -9.638  1.00 39.43          N
ATOM   3353  CA   LYS A 752      30.769  -8.341  -8.658  1.00 38.83          C
ATOM   3355  CB   LYS A 752      29.564  -9.302  -8.911  1.00 39.25          C
ATOM   3358  CG   LYS A 752      28.455  -9.313  -7.841  1.00 38.89          C
ATOM   3361  CD   LYS A 752      27.855  -7.931  -7.595  1.00 39.51          C
ATOM   3364  CE   LYS A 752      26.957  -7.457  -8.782  1.00 38.66          C
ATOM   3367  NZ   LYS A 752      25.767  -8.330  -8.981  1.00 38.40          N
ATOM   3371  C    LYS A 752      31.267  -8.492  -7.246  1.00 38.37          C
ATOM   3372  O    LYS A 752      31.015  -7.605  -6.402  1.00 38.69          O
ATOM   3373  N    GLU A 753      31.947  -9.599  -6.947  1.00 38.00          N
ATOM   3375  CA   GLU A 753      32.392  -9.855  -5.574  1.00 39.11          C
ATOM   3377  CB   GLU A 753      33.066 -11.235  -5.422  1.00 40.06          C
ATOM   3380  CG   GLU A 753      32.071 -12.421  -5.462  1.00 43.59          C
ATOM   3383  CD   GLU A 753      32.680 -13.799  -5.799  1.00 48.13          C
ATOM   3384  OE1  GLU A 753      33.907 -13.899  -6.081  1.00 51.83          O
ATOM   3385  OE2  GLU A 753      31.910 -14.806  -5.784  1.00 48.81          O
ATOM   3386  C    GLU A 753      33.328  -8.705  -5.159  1.00 38.39          C
ATOM   3387  O    GLU A 753      33.232  -8.168  -4.074  1.00 37.95          O
ATOM   3388  N    LEU A 754      34.154  -8.304  -6.110  1.00 37.59          N
ATOM   3390  CA   LEU A 754      35.181  -7.295  -5.950  1.00 37.06          C
ATOM   3392  CB   LEU A 754      35.993  -7.307  -7.233  1.00 36.87          C
ATOM   3395  CG   LEU A 754      37.238  -6.492  -7.427  1.00 39.55          C
ATOM   3397  CD1  LEU A 754      36.787  -5.122  -7.839  1.00 42.06          C
ATOM   3401  CD2  LEU A 754      38.169  -6.458  -6.171  1.00 41.44          C
ATOM   3405  C    LEU A 754      34.575  -5.908  -5.693  1.00 36.10          C
ATOM   3406  O    LEU A 754      35.066  -5.140  -4.862  1.00 33.82          O
ATOM   3407  N    PHE A 755      33.517  -5.593  -6.427  1.00 35.18          N
ATOM   3409  CA   PHE A 755      32.812  -4.320  -6.260  1.00 34.80          C
ATOM   3411  CB   PHE A 755      31.773  -4.066  -7.365  1.00 35.11          C
ATOM   3414  CG   PHE A 755      30.958  -2.838  -7.115  1.00 36.68          C
ATOM   3415  CD1  PHE A 755      31.570  -1.598  -7.077  1.00 39.95          C
ATOM   3417  CE1  PHE A 755      30.861  -0.476  -6.794  1.00 41.31          C
ATOM   3419  CZ   PHE A 755      29.522  -0.565  -6.523  1.00 40.36          C
ATOM   3421  CE2  PHE A 755      28.899  -1.780  -6.553  1.00 40.16          C
ATOM   3423  CD2  PHE A 755      29.624  -2.921  -6.821  1.00 39.43          C
ATOM   3425  C    PHE A 755      32.126  -4.275  -4.907  1.00 33.82          C
ATOM   3426  O    PHE A 755      32.186  -3.268  -4.222  1.00 34.54          O
ATOM   3427  N    LEU A 756      31.488  -5.365  -4.510  1.00 33.07          N
ATOM   3429  CA   LEU A 756      30.889  -5.452  -3.190  1.00 33.07          C
ATOM   3431  CB   LEU A 756      30.198  -6.810  -2.988  1.00 33.19          C
ATOM   3434  CG   LEU A 756      28.965  -7.139  -3.870  1.00 35.16          C
ATOM   3436  CD1  LEU A 756      28.482  -8.535  -3.574  1.00 37.25          C
ATOM   3440  CD2  LEU A 756      27.831  -6.214  -3.702  1.00 35.98          C
ATOM   3444  C    LEU A 756      31.937  -5.203  -2.074  1.00 33.15          C
ATOM   3445  O    LEU A 756      31.626  -4.600  -1.017  1.00 31.74          O
ATOM   3446  N    ALA A 757      33.166  -5.682  -2.309  1.00 33.12          N
ATOM   3448  CA   ALA A 757      34.238  -5.537  -1.323  1.00 33.06          C
ATOM   3450  CB   ALA A 757      35.395  -6.482  -1.623  1.00 32.92          C
ATOM   3454  C    ALA A 757      34.712  -4.091  -1.269  1.00 33.03          C
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ATOM   3455  O    ALA A 757     34.882  -3.542  -0.176  1.00 34.12           O
ATOM   3456  N    MET A 758     34.864  -3.452  -2.424  1.00 32.95           N
ATOM   3458  CA   MET A 758     35.271  -2.049  -2.457  1.00 33.93           C
ATOM   3460  CB   MET A 758     35.527  -1.574  -3.881  1.00 33.91           C
ATOM   3463  CG   MET A 758     36.672  -2.314  -4.621  1.00 35.83           C
ATOM   3466  SD   MET A 758     38.384  -2.046  -3.916  1.00 38.11           S
ATOM   3467  CE   MET A 758     38.783  -3.635  -3.324  1.00 37.96           C
ATOM   3471  C    MET A 758     34.213  -1.167  -1.771  1.00 34.32           C
ATOM   3472  O    MET A 758     34.548  -0.214  -1.088  1.00 33.57           O
ATOM   3473  N    LEU A 759     32.937  -1.517  -1.934  1.00 35.01           N
ATOM   3475  CA   LEU A 759     31.833  -0.785  -1.311  1.00 35.47           C
ATOM   3477  CB   LEU A 759     30.519  -1.275  -1.903  1.00 35.61           C
ATOM   3480  CG   LEU A 759     29.229  -0.558  -1.509  1.00 38.34           C
ATOM   3482  CD1  LEU A 759     29.323   0.977  -1.635  1.00 38.93           C
ATOM   3486  CD2  LEU A 759     28.107  -1.116  -2.384  1.00 38.44           C
ATOM   3490  C    LEU A 759     31.801  -0.916   0.214  1.00 35.04           C
ATOM   3491  O    LEU A 759     31.481   0.038   0.926  1.00 35.94           O
ATOM   3492  N    MET A 760     32.084  -2.115   0.715  1.00 34.32           N
ATOM   3494  CA   MET A 760     32.197  -2.345   2.140  1.00 33.02           C
ATOM   3496  CB   MET A 760     32.522  -3.821   2.427  1.00 33.34           C
ATOM   3499  CG   MET A 760     31.323  -4.760   2.332  1.00 32.73           C
ATOM   3502  SD   MET A 760     29.985  -4.481   3.492  1.00 35.30           S
ATOM   3503  CE   MET A 760     30.754  -4.506   5.105  1.00 33.09           C
ATOM   3507  C    MET A 760     33.293  -1.448   2.691  1.00 32.13           C
ATOM   3508  O    MET A 760     33.105  -0.774   3.689  1.00 31.76           O
ATOM   3509  N    THR A 761     34.422  -1.403   2.000  1.00 31.96           N
ATOM   3511  CA   THR A 761     35.526  -0.561   2.431  1.00 31.48           C
ATOM   3513  CB   THR A 761     36.770  -0.755   1.568  1.00 30.73           C
ATOM   3515  OG1  THR A 761     37.198  -2.120   1.636  1.00 28.72           O
ATOM   3517  CG2  THR A 761     37.929   0.055   2.153  1.00 30.78           C
ATOM   3521  C    THR A 761     35.127   0.897   2.445  1.00 31.80           C
ATOM   3522  O    THR A 761     35.411   1.597   3.418  1.00 31.67           O
ATOM   3523  N    ALA A 762     34.438   1.338   1.395  1.00 31.82           N
ATOM   3525  CA   ALA A 762     34.028   2.740   1.254  1.00 32.31           C
ATOM   3527  CB   ALA A 762     33.340   2.968  -0.094  1.00 32.30           C
ATOM   3531  C    ALA A 762     33.110   3.157   2.388  1.00 32.01           C
ATOM   3532  O    ALA A 762     33.268   4.251   2.954  1.00 32.18           O
ATOM   3533  N    CYS A 763     32.198   2.256   2.772  1.00 31.80           N
ATOM   3535  CA   CYS A 763     31.290   2.513   3.913  1.00 31.62           C
ATOM   3537  CB   CYS A 763     30.127   1.541   3.891  1.00 31.53           C
ATOM   3540  SG   CYS A 763     29.097   1.770   2.386  1.00 33.88           S
ATOM   3541  C    CYS A 763     31.986   2.460   5.278  1.00 31.47           C
ATOM   3542  O    CYS A 763     31.705   3.252   6.142  1.00 32.16           O
ATOM   3543  N    ASP A 764     32.915   1.529   5.435  1.00 32.05           N
ATOM   3545  CA   ASP A 764     33.661   1.338   6.657  1.00 32.31           C
ATOM   3547  CB   ASP A 764     34.548   0.107   6.478  1.00 32.85           C
ATOM   3550  CG   ASP A 764     34.997  -0.484   7.793  1.00 32.98           C
ATOM   3551  OD1  ASP A 764     34.339  -0.212   8.822  1.00 35.64           O
ATOM   3552  OD2  ASP A 764     36.023  -1.205   7.874  1.00 34.26           O
ATOM   3553  C    ASP A 764     34.536   2.559   7.006  1.00 32.69           C
ATOM   3554  O    ASP A 764     34.684   2.894   8.176  1.00 32.86           O
ATOM   3555  N    LEU A 765     35.082   3.221   5.979  1.00 32.87           N
ATOM   3557  CA   LEU A 765     36.021   4.343   6.156  1.00 33.11           C
ATOM   3559  CB   LEU A 765     37.121   4.304   5.073  1.00 32.59           C
ATOM   3562  CG   LEU A 765     37.940   3.020   4.974  1.00 35.12           C
ATOM   3564  CD1  LEU A 765     39.050   3.150   3.927  1.00 34.93           C
ATOM   3568  CD2  LEU A 765     38.559   2.603   6.310  1.00 36.80           C
ATOM   3572  C    LEU A 765     35.337   5.698   6.085  1.00 32.04           C
ATOM   3573  O    LEU A 765     35.994   6.720   6.171  1.00 31.39           O
ATOM   3574  N    SER A 766     34.015   5.704   5.940  1.00 32.14           N
ATOM   3576  CA   SER A 766     33.288   6.902   5.555  1.00 31.82           C
ATOM   3578  CB   SER A 766     31.860   6.549   5.094  1.00 32.63           C
ATOM   3581  OG   SER A 766     31.110   5.983   6.153  1.00 32.46           O
ATOM   3583  C    SER A 766     33.230   8.033   6.590  1.00 31.12           C
ATOM   3584  O    SER A 766     32.846   9.120   6.226  1.00 30.64           O
ATOM   3585  N    ALA A 767     33.600   7.802   7.851  1.00 30.80           N
ATOM   3587  CA   ALA A 767     33.854   8.938   8.767  1.00 30.32           C
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ATOM   3589  CB   ALA A 767      34.492    8.458   10.078  1.00 30.27           C
ATOM   3593  C    ALA A 767      34.763    9.988    8.092  1.00 29.89           C
ATOM   3594  O    ALA A 767      34.628   11.170    8.343  1.00 29.21           O
ATOM   3595  N    ILE A 768      35.694    9.538    7.239  1.00 30.00           N
ATOM   3597  CA   ILE A 768      36.646   10.422    6.569  1.00 30.23           C
ATOM   3599  CB   ILE A 768      37.817    9.582    5.948  1.00 30.76           C
ATOM   3601  CG1  ILE A 768      39.050   10.423    5.684  1.00 32.05           C
ATOM   3604  CD1  ILE A 768      39.698   11.003    6.933  1.00 35.25           C
ATOM   3608  CG2  ILE A 768      37.413    9.018    4.601  1.00 32.10           C
ATOM   3612  C    ILE A 768      35.999   11.349    5.524  1.00 30.34           C
ATOM   3613  O    ILE A 768      36.625   12.337    5.070  1.00 30.09           O
ATOM   3614  N    THR A 769      34.770   11.048    5.125  1.00 30.53           N
ATOM   3616  CA   THR A 769      34.037   11.910    4.186  1.00 31.36           C
ATOM   3618  CB   THR A 769      33.212   11.057    3.209  1.00 31.66           C
ATOM   3620  OG1  THR A 769      32.133   10.411    3.905  1.00 31.66           O
ATOM   3622  CG2  THR A 769      34.031    9.921    2.660  1.00 31.10           C
ATOM   3626  C    THR A 769      33.087   12.926    4.840  1.00 31.89           C
ATOM   3627  O    THR A 769      32.474   13.732    4.144  1.00 32.05           O
ATOM   3628  N    LYS A 770      32.976   12.908    6.164  1.00 31.96           N
ATOM   3630  CA   LYS A 770      31.939   13.694    6.859  1.00 31.48           C
ATOM   3632  CB   LYS A 770      31.757   13.185    8.300  1.00 31.33           C
ATOM   3635  CG   LYS A 770      31.213   11.754    8.406  1.00 31.96           C
ATOM   3638  CD   LYS A 770      29.743   11.641    8.063  1.00 33.71           C
ATOM   3641  CE   LYS A 770      29.300   10.166    8.044  1.00 35.91           C
ATOM   3644  NZ   LYS A 770      27.964    9.987    7.459  1.00 35.13           N
ATOM   3648  C    LYS A 770      32.251   15.172    6.867  1.00 30.72           C
ATOM   3649  O    LYS A 770      33.400   15.549    6.735  1.00 31.50           O
ATOM   3650  N    PRO A 771      31.243   16.034    7.036  1.00 30.88           N
ATOM   3651  CA   PRO A 771      31.515   17.472    7.157  1.00 30.34           C
ATOM   3653  CB   PRO A 771      30.162   18.066    7.577  1.00 30.70           C
ATOM   3656  CG   PRO A 771      29.144   17.093    7.075  1.00 29.87           C
ATOM   3659  CD   PRO A 771      29.801   15.730    7.154  1.00 30.49           C
ATOM   3662  C    PRO A 771      32.578   17.678    8.234  1.00 30.56           C
ATOM   3663  O    PRO A 771      32.621   16.901    9.192  1.00 30.01           O
ATOM   3664  N    TRP A 772      33.415   18.690    8.067  1.00 30.74           N
ATOM   3666  CA   TRP A 772      34.580   18.907    8.922  1.00 31.54           C
ATOM   3668  CB   TRP A 772      35.215   20.260    8.605  1.00 31.15           C
ATOM   3671  CG   TRP A 772      36.310   20.628    9.554  1.00 31.74           C
ATOM   3672  CD1  TRP A 772      36.346   21.706   10.395  1.00 30.69           C
ATOM   3674  NE1  TRP A 772      37.514   21.708   11.119  1.00 30.64           N
ATOM   3676  CE2  TRP A 772      38.252   20.610   10.766  1.00 32.70           C
ATOM   3677  CD2  TRP A 772      37.525   19.911    9.777  1.00 31.92           C
ATOM   3678  CE3  TRP A 772      38.075   18.741    9.247  1.00 33.06           C
ATOM   3680  CZ3  TRP A 772      39.310   18.315    9.700  1.00 32.82           C
ATOM   3682  CH2  TRP A 772      40.017   19.042   10.664  1.00 33.20           C
ATOM   3684  CZ2  TRP A 772      39.505   20.191   11.210  1.00 32.99           C
ATOM   3686  C    TRP A 772      34.370   18.772   10.448  1.00 32.29           C
ATOM   3687  O    TRP A 772      35.158   18.085   11.082  1.00 32.63           O
ATOM   3688  N    PRO A 773      33.353   19.406   11.045  1.00 32.84           N
ATOM   3689  CA   PRO A 773      33.174   19.318   12.505  1.00 33.39           C
ATOM   3691  CB   PRO A 773      31.938   20.202   12.785  1.00 33.59           C
ATOM   3694  CG   PRO A 773      31.831   21.109   11.595  1.00 33.72           C
ATOM   3697  CD   PRO A 773      32.313   20.241   10.420  1.00 32.94           C
ATOM   3700  C    PRO A 773      32.932   17.897   13.000  1.00 33.89           C
ATOM   3701  O    PRO A 773      33.352   17.574   14.108  1.00 34.70           O
ATOM   3702  N    ILE A 774      32.275   17.069   12.200  1.00 33.06           N
ATOM   3704  CA   ILE A 774      32.049   15.689   12.561  1.00 33.95           C
ATOM   3706  CB   ILE A 774      30.906   15.131   11.696  1.00 34.28           C
ATOM   3708  CG1  ILE A 774      29.592   15.855   12.055  1.00 36.56           C
ATOM   3711  CD1  ILE A 774      28.580   15.882   10.928  1.00 37.65           C
ATOM   3715  CG2  ILE A 774      30.780   13.606   11.848  1.00 34.99           C
ATOM   3719  C    ILE A 774      33.317   14.831   12.421  1.00 34.03           C
ATOM   3720  O    ILE A 774      33.637   14.032   13.311  1.00 34.05           O
ATOM   3721  N    GLN A 775      34.029   14.991   11.305  1.00 33.53           N
ATOM   3723  CA   GLN A 775      35.276   14.259   11.075  1.00 33.26           C
ATOM   3725  CB   GLN A 775      35.781   14.483    9.630  1.00 32.88           C
ATOM   3728  CG   GLN A 775      37.294   14.320    9.411  1.00 32.21           C
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ATOM    3731   CD   GLN A 775      37.819  12.966   9.789  1.00 29.57           C
ATOM    3732   OE1  GLN A 775      37.072  11.968   9.828  1.00 32.23           O
ATOM    3733   NE2  GLN A 775      39.108  12.904  10.058  1.00 27.57           N
ATOM    3736   C    GLN A 775      36.334  14.620  12.138  1.00 33.48           C
ATOM    3737   O    GLN A 775      36.931  13.721  12.725  1.00 33.59           O
ATOM    3738   N    GLN A 776      36.553  15.912  12.389  1.00 33.83           N
ATOM    3740   CA   GLN A 776      37.544  16.350  13.391  1.00 34.39           C
ATOM    3742   CB   GLN A 776      37.540  17.866  13.599  1.00 34.52           C
ATOM    3745   CG   GLN A 776      38.810  18.396  14.333  1.00 35.28           C
ATOM    3748   CD   GLN A 776      38.898  19.924  14.435  1.00 35.65           C
ATOM    3749   OE1  GLN A 776      39.982  20.490  14.355  1.00 35.83           O
ATOM    3750   NE2  GLN A 776      37.767  20.577  14.641  1.00 37.48           N
ATOM    3753   C    GLN A 776      37.254  15.648  14.709  1.00 35.11           C
ATOM    3754   O    GLN A 776      38.138  15.040  15.306  1.00 34.23           O
ATOM    3755   N    ARG A 777      35.989  15.710  15.120  1.00 35.48           N
ATOM    3757   CA   ARG A 777      35.524  15.082  16.349  1.00 36.93           C
ATOM    3759   CB   ARG A 777      34.033  15.360  16.534  1.00 37.68           C
ATOM    3762   CG   ARG A 777      33.637  15.541  17.927  1.00 42.37           C
ATOM    3765   CD   ARG A 777      33.662  14.250  18.764  1.00 47.31           C
ATOM    3768   NE   ARG A 777      33.889  14.588  20.177  1.00 50.43           N
ATOM    3770   CZ   ARG A 777      32.935  14.780  21.062  1.00 49.65           C
ATOM    3771   NH1  ARG A 777      31.654  14.659  20.708  1.00 51.41           N
ATOM    3774   NH2  ARG A 777      33.270  15.086  22.304  1.00 49.88           N
ATOM    3777   C    ARG A 777      35.747  13.568  16.411  1.00 36.41           C
ATOM    3778   O    ARG A 777      36.304  13.087  17.386  1.00 36.09           O
ATOM    3779   N    ILE A 778      35.291  12.826  15.401  1.00 35.82           N
ATOM    3781   CA   ILE A 778      35.476  11.377  15.379  1.00 36.75           C
ATOM    3783   CB   ILE A 778      34.957  10.723  14.057  1.00 36.93           C
ATOM    3785   CG1  ILE A 778      33.437  10.792  13.978  1.00 39.16           C
ATOM    3788   CD1  ILE A 778      32.868  10.202  12.692  1.00 40.52           C
ATOM    3792   CG2  ILE A 778      35.355   9.247  14.011  1.00 39.29           C
ATOM    3796   C    ILE A 778      36.944  11.027  15.497  1.00 35.85           C
ATOM    3797   O    ILE A 778      37.312  10.105  16.244  1.00 35.82           O
ATOM    3798   N    ALA A 779      37.765  11.754  14.730  1.00 34.32           N
ATOM    3800   CA   ALA A 779      39.186  11.455  14.599  1.00 33.17           C
ATOM    3802   CB   ALA A 779      39.824  12.324  13.505  1.00 32.80           C
ATOM    3806   C    ALA A 779      39.854  11.697  15.947  1.00 32.84           C
ATOM    3807   O    ALA A 779      40.703  10.928  16.366  1.00 31.43           O
ATOM    3808   N    GLU A 780      39.430  12.750  16.645  1.00 32.74           N
ATOM    3810   CA   GLU A 780      39.957  13.022  17.982  1.00 33.52           C
ATOM    3812   CB   GLU A 780      39.479  14.371  18.519  1.00 34.36           C
ATOM    3815   CG   GLU A 780      39.675  15.507  17.542  1.00 37.36           C
ATOM    3818   CD   GLU A 780      40.731  16.458  17.945  1.00 42.43           C
ATOM    3819   OE1  GLU A 780      40.376  17.546  18.475  1.00 44.44           O
ATOM    3820   OE2  GLU A 780      41.913  16.111  17.675  1.00 47.53           O
ATOM    3821   C    GLU A 780      39.587  11.965  18.986  1.00 32.66           C
ATOM    3822   O    GLU A 780      40.396  11.621  19.829  1.00 32.54           O
ATOM    3823   N    LEU A 781      38.360  11.471  18.904  1.00 32.94           N
ATOM    3825   CA   LEU A 781      37.849  10.459  19.841  1.00 33.38           C
ATOM    3827   CB   LEU A 781      36.356  10.264  19.662  1.00 33.14           C
ATOM    3830   CG   LEU A 781      35.463  11.268  20.393  1.00 37.00           C
ATOM    3832   CD1  LEU A 781      34.009  10.910  20.097  1.00 40.21           C
ATOM    3836   CD2  LEU A 781      35.731  11.316  21.899  1.00 37.56           C
ATOM    3840   C    LEU A 781      38.558   9.140  19.623  1.00 33.33           C
ATOM    3841   O    LEU A 781      39.014   8.479  20.568  1.00 33.55           O
ATOM    3842   N    VAL A 782      38.689   8.810  18.354  1.00 33.23           N
ATOM    3844   CA   VAL A 782      39.315   7.586  17.916  1.00 34.70           C
ATOM    3846   CB   VAL A 782      39.143   7.435  16.382  1.00 35.07           C
ATOM    3848   CG1  VAL A 782      40.142   6.493  15.802  1.00 37.35           C
ATOM    3852   CG2  VAL A 782      37.706   7.020  16.073  1.00 34.78           C
ATOM    3856   C    VAL A 782      40.758   7.578  18.352  1.00 34.45           C
ATOM    3857   O    VAL A 782      41.237   6.597  18.933  1.00 35.02           O
ATOM    3858   N    ALA A 783      41.425   8.711  18.158  1.00 34.54           N
ATOM    3860   CA   ALA A 783      42.802   8.854  18.570  1.00 34.07           C
ATOM    3862   CB   ALA A 783      43.366  10.195  18.084  1.00 34.19           C
ATOM    3866   C    ALA A 783      42.940   8.715  20.077  1.00 33.96           C
ATOM    3867   O    ALA A 783      43.844   8.055  20.563  1.00 34.29           O
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ATOM   3868   N    THR A 784    42.061    9.362   20.829   1.00 33.54        N
ATOM   3870   CA   THR A 784    42.079    9.254   22.284   1.00 33.02        C
ATOM   3872   CB   THR A 784    40.985   10.163   22.882   1.00 33.03        C
ATOM   3874   OG1  THR A 784    41.372   11.520   22.687   1.00 33.63        O
ATOM   3876   CG2  THR A 784    40.873    9.995   24.372   1.00 34.14        C
ATOM   3880   C    THR A 784    41.836    7.808   22.713   1.00 32.12        C
ATOM   3881   O    THR A 784    42.523    7.293   23.585   1.00 30.96        O
ATOM   3882   N    GLU A 785    40.881    7.150   22.073   1.00 31.76        N
ATOM   3884   CA   GLU A 785    40.597    5.763   22.421   1.00 32.72        C
ATOM   3886   CB   GLU A 785    39.424    5.240   21.629   1.00 33.09        C
ATOM   3889   CG   GLU A 785    38.120    5.797   22.173   1.00 35.55        C
ATOM   3892   CD   GLU A 785    37.114    6.142   21.097   1.00 39.51        C
ATOM   3893   OE1  GLU A 785    37.160    5.569   19.956   1.00 37.48        O
ATOM   3894   OE2  GLU A 785    36.253    6.992   21.418   1.00 40.23        O
ATOM   3895   C    GLU A 785    41.823    4.862   22.270   1.00 32.40        C
ATOM   3896   O    GLU A 785    42.063    4.000   23.121   1.00 32.01        O
ATOM   3897   N    PHE A 786    42.637    5.090   21.236   1.00 31.75        N
ATOM   3899   CA   PHE A 786    43.821    4.266   21.052   1.00 31.08        C
ATOM   3901   CB   PHE A 786    44.302    4.254   19.589   1.00 30.76        C
ATOM   3904   CG   PHE A 786    45.638    3.603   19.430   1.00 28.95        C
ATOM   3905   CD1  PHE A 786    45.733    2.227   19.231   1.00 28.19        C
ATOM   3907   CE1  PHE A 786    46.966    1.598   19.126   1.00 26.34        C
ATOM   3909   CZ   PHE A 786    48.124    2.354   19.193   1.00 28.48        C
ATOM   3911   CE2  PHE A 786    48.046    3.737   19.396   1.00 29.04        C
ATOM   3913   CD2  PHE A 786    46.800    4.352   19.506   1.00 27.06        C
ATOM   3915   C    PHE A 786    44.954    4.709   21.954   1.00 31.42        C
ATOM   3916   O    PHE A 786    45.556    3.899   22.664   1.00 31.10        O
ATOM   3917   N    PHE A 787    45.274    5.997   21.904   1.00 31.90        N
ATOM   3919   CA   PHE A 787    46.485    6.498   22.527   1.00 33.00        C
ATOM   3921   CB   PHE A 787    46.832    7.920   22.033   1.00 32.94        C
ATOM   3924   CG   PHE A 787    47.416    7.944   20.647   1.00 33.16        C
ATOM   3925   CD1  PHE A 787    46.739    8.554   19.592   1.00 34.71        C
ATOM   3927   CE1  PHE A 787    47.260    8.551   18.300   1.00 34.34        C
ATOM   3929   CZ   PHE A 787    48.445    7.937   18.057   1.00 32.95        C
ATOM   3931   CE2  PHE A 787    49.141    7.319   19.119   1.00 33.74        C
ATOM   3933   CD2  PHE A 787    48.616    7.315   20.387   1.00 31.79        C
ATOM   3935   C    PHE A 787    46.451    6.431   24.058   1.00 34.24        C
ATOM   3936   O    PHE A 787    47.504    6.333   24.660   1.00 33.67        O
ATOM   3937   N    ASP A 788    45.261    6.426   24.659   1.00 35.47        N
ATOM   3939   CA   ASP A 788    45.120    6.375   26.118   1.00 37.43        C
ATOM   3941   CB   ASP A 788    44.295    7.571   26.622   1.00 37.71        C
ATOM   3944   CG   ASP A 788    44.916    8.910   26.261   1.00 39.74        C
ATOM   3945   OD1  ASP A 788    46.162    9.028   26.237   1.00 41.13        O
ATOM   3946   OD2  ASP A 788    44.226    9.908   25.985   1.00 44.69        O
ATOM   3947   C    ASP A 788    44.430    5.097   26.586   1.00 38.24        C
ATOM   3948   O    ASP A 788    43.745    5.111   27.604   1.00 39.37        O
ATOM   3949   N    GLN A 789    44.604    3.998   25.856   1.00 38.19        N
ATOM   3951   CA   GLN A 789    43.942    2.744   26.211   1.00 38.18        C
ATOM   3953   CB   GLN A 789    43.888    1.806   24.985   1.00 37.38        C
ATOM   3956   CG   GLN A 789    45.238    1.289   24.523   1.00 36.46        C
ATOM   3959   CD   GLN A 789    45.095    0.409   23.304   1.00 36.18        C
ATOM   3960   OE1  GLN A 789    45.349    0.839   22.152   1.00 34.24        O
ATOM   3961   NE2  GLN A 789    44.654   -0.815   23.536   1.00 30.76        N
ATOM   3964   C    GLN A 789    44.563    2.029   27.439   1.00 38.14        C
ATOM   3965   O    GLN A 789    45.646    2.404   27.938   1.00 39.39        O
ATOM   3966   N    LEU A 804    47.292   14.102   30.667   1.00 48.20        N
ATOM   3968   CA   LEU A 804    48.025   15.289   31.085   1.00 48.23        C
ATOM   3970   CB   LEU A 804    48.616   15.100   32.503   1.00 48.00        C
ATOM   3973   CG   LEU A 804    47.806   15.491   33.769   1.00 46.92        C
ATOM   3975   CD1  LEU A 804    48.696   15.513   35.023   1.00 45.99        C
ATOM   3979   CD2  LEU A 804    47.075   16.830   33.646   1.00 46.22        C
ATOM   3983   C    LEU A 804    49.131   15.673   30.083   1.00 48.83        C
ATOM   3984   O    LEU A 804    50.011   16.473   30.414   1.00 48.85        O
ATOM   3985   N    MET A 805    49.098   15.113   28.869   1.00 49.61        N
ATOM   3987   CA   MET A 805    49.982   15.606   27.787   1.00 50.21        C
ATOM   3989   CB   MET A 805    50.983   14.551   27.265   1.00 50.52        C
ATOM   3992   CG   MET A 805    50.399   13.177   26.872   1.00 52.14        C
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ATOM	3995	SD	MET	A	805	51.017	11.863	27.954	1.00 55.69	S
ATOM	3996	CE	MET	A	805	52.683	11.540	27.248	1.00 53.87	C
ATOM	4000	C	MET	A	805	49.149	16.213	26.649	1.00 49.81	C
ATOM	4001	O	MET	A	805	49.319	17.404	26.356	1.00 50.89	O
ATOM	4002	N	ASN	A	806	48.271	15.410	26.030	1.00 48.96	N
ATOM	4004	CA	ASN	A	806	47.228	15.890	25.111	1.00 48.12	C
ATOM	4006	CB	ASN	A	806	46.341	16.948	25.794	1.00 48.40	C
ATOM	4009	CG	ASN	A	806	45.306	16.328	26.687	1.00 48.99	C
ATOM	4010	OD1	ASN	A	806	44.550	15.469	26.243	1.00 48.89	O
ATOM	4011	ND2	ASN	A	806	45.286	16.730	27.963	1.00 48.58	N
ATOM	4014	C	ASN	A	806	47.739	16.454	23.804	1.00 46.98	C
ATOM	4015	O	ASN	A	806	47.348	15.982	22.743	1.00 46.74	O
ATOM	4016	N	ARG	A	807	48.504	17.540	23.912	1.00 45.71	N
ATOM	4018	CA	ARG	A	807	49.315	18.096	22.837	1.00 44.96	C
ATOM	4020	CB	ARG	A	807	50.422	18.984	23.418	1.00 44.75	C
ATOM	4023	CG	ARG	A	807	49.988	20.372	23.859	1.00 44.98	C
ATOM	4026	CD	ARG	A	807	51.038	21.442	23.571	1.00 44.24	C
ATOM	4029	NE	ARG	A	807	51.043	22.518	24.553	1.00 44.44	N
ATOM	4031	CZ	ARG	A	807	51.802	23.608	24.469	1.00 44.47	C
ATOM	4032	NH1	ARG	A	807	52.636	23.781	23.445	1.00 44.73	N
ATOM	4035	NH2	ARG	A	807	51.736	24.535	25.419	1.00 44.86	N
ATOM	4038	C	ARG	A	807	49.977	17.018	22.007	1.00 44.15	C
ATOM	4039	O	ARG	A	807	49.721	16.930	20.817	1.00 44.46	O
ATOM	4040	N	GLU	A	808	50.823	16.206	22.640	1.00 43.47	N
ATOM	4042	CA	GLU	A	808	51.549	15.127	21.952	1.00 43.32	C
ATOM	4044	CB	GLU	A	808	52.339	14.273	22.962	1.00 43.39	C
ATOM	4047	CG	GLU	A	808	53.743	14.811	23.263	1.00 44.43	C
ATOM	4050	CD	GLU	A	808	54.087	14.859	24.752	1.00 46.76	C
ATOM	4051	OE1	GLU	A	808	53.612	13.988	25.518	1.00 49.69	O
ATOM	4052	OE2	GLU	A	808	54.847	15.768	25.166	1.00 46.32	O
ATOM	4053	C	GLU	A	808	50.621	14.252	21.100	1.00 42.71	C
ATOM	4054	O	GLU	A	808	50.993	13.807	20.015	1.00 43.71	O
ATOM	4055	N	LYS	A	809	49.411	14.028	21.591	1.00 41.53	N
ATOM	4057	CA	LYS	A	809	48.393	13.268	20.879	1.00 41.25	C
ATOM	4059	CB	LYS	A	809	47.240	12.930	21.861	1.00 41.18	C
ATOM	4062	CG	LYS	A	809	46.242	11.871	21.390	1.00 40.13	C
ATOM	4065	CD	LYS	A	809	44.800	12.047	21.921	1.00 39.55	C
ATOM	4068	CE	LYS	A	809	44.702	12.763	23.279	1.00 41.48	C
ATOM	4071	NZ	LYS	A	809	43.352	12.612	23.881	1.00 42.05	N
ATOM	4075	C	LYS	A	809	47.854	14.078	19.692	1.00 40.56	C
ATOM	4076	O	LYS	A	809	47.896	13.638	18.547	1.00 40.59	O
ATOM	4077	N	LYS	A	810	47.353	15.270	19.996	1.00 39.94	N
ATOM	4079	CA	LYS	A	810	46.595	16.086	19.056	1.00 39.38	C
ATOM	4081	CB	LYS	A	810	46.030	17.334	19.750	1.00 39.37	C
ATOM	4084	CG	LYS	A	810	44.595	17.168	20.259	1.00 41.26	C
ATOM	4087	CD	LYS	A	810	44.144	18.418	21.043	1.00 43.79	C
ATOM	4090	CE	LYS	A	810	42.631	18.636	20.928	1.00 44.96	C
ATOM	4093	NZ	LYS	A	810	42.290	20.033	21.357	1.00 46.60	N
ATOM	4097	C	LYS	A	810	47.447	16.501	17.871	1.00 38.49	C
ATOM	4098	O	LYS	A	810	46.935	16.581	16.755	1.00 38.38	O
ATOM	4099	N	ASN	A	811	48.728	16.756	18.138	1.00 37.29	N
ATOM	4101	CA	ASN	A	811	49.707	17.123	17.120	1.00 37.08	C
ATOM	4103	CB	ASN	A	811	51.015	17.581	17.771	1.00 36.70	C
ATOM	4106	CG	ASN	A	811	50.949	19.013	18.267	1.00 38.21	C
ATOM	4107	OD1	ASN	A	811	50.010	19.751	17.962	1.00 40.42	O
ATOM	4108	ND2	ASN	A	811	51.951	19.415	19.039	1.00 36.98	N
ATOM	4111	C	ASN	A	811	50.029	16.029	16.094	1.00 36.40	C
ATOM	4112	O	ASN	A	811	50.519	16.338	15.020	1.00 36.71	O
ATOM	4113	N	LYS	A	812	49.794	14.765	16.443	1.00 35.59	N
ATOM	4115	CA	LYS	A	812	49.952	13.658	15.515	1.00 34.80	C
ATOM	4117	CB	LYS	A	812	49.997	12.321	16.261	1.00 35.36	C
ATOM	4120	CG	LYS	A	812	51.178	12.089	17.197	1.00 38.10	C
ATOM	4123	CD	LYS	A	812	50.943	10.811	18.045	1.00 40.15	C
ATOM	4126	CE	LYS	A	812	52.167	10.495	18.955	1.00 42.19	C
ATOM	4129	NZ	LYS	A	812	51.729	10.181	20.375	1.00 43.84	N
ATOM	4133	C	LYS	A	812	48.809	13.565	14.505	1.00 33.50	C
ATOM	4134	O	LYS	A	812	49.002	13.066	13.399	1.00 33.42	O

ATOM	4135	N	ILE	A	813	47.612	13.997	14.885	1.00	31.89	N
ATOM	4137	CA	ILE	A	813	46.419	13.628	14.139	1.00	31.20	C
ATOM	4139	CB	ILE	A	813	45.112	13.971	14.923	1.00	31.60	C
ATOM	4141	CG1	ILE	A	813	45.053	13.185	16.235	1.00	31.56	C
ATOM	4144	CD1	ILE	A	813	43.972	13.688	17.154	1.00	33.08	C
ATOM	4148	CG2	ILE	A	813	43.856	13.633	14.078	1.00	31.70	C
ATOM	4152	C	ILE	A	813	46.335	14.128	12.685	1.00	30.20	C
ATOM	4153	O	ILE	A	813	45.916	13.360	11.822	1.00	29.75	O
ATOM	4154	N	PRO	A	814	46.641	15.400	12.420	1.00	29.48	N
ATOM	4155	CA	PRO	A	814	46.591	15.922	11.047	1.00	29.05	C
ATOM	4157	CB	PRO	A	814	47.109	17.351	11.195	1.00	29.41	C
ATOM	4160	CG	PRO	A	814	46.719	17.725	12.636	1.00	28.96	C
ATOM	4163	CD	PRO	A	814	46.977	16.463	13.391	1.00	29.10	C
ATOM	4166	C	PRO	A	814	47.414	15.109	10.049	1.00	28.97	C
ATOM	4167	O	PRO	A	814	46.860	14.740	9.019	1.00	28.23	O
ATOM	4168	N	SER	A	815	48.653	14.778	10.377	1.00	28.56	N
ATOM	4170	CA	SER	A	815	49.506	14.002	9.462	1.00	29.09	C
ATOM	4172	CB	SER	A	815	50.962	13.975	9.927	1.00	28.44	C
ATOM	4175	OG	SER	A	815	51.511	15.248	9.770	1.00	32.41	O
ATOM	4177	C	SER	A	815	49.001	12.586	9.329	1.00	28.45	C
ATOM	4178	O	SER	A	815	49.115	12.000	8.246	1.00	27.40	O
ATOM	4179	N	MET	A	816	48.443	12.039	10.417	1.00	27.90	N
ATOM	4181	CA	MET	A	816	47.820	10.719	10.346	1.00	27.88	C
ATOM	4183	CB	MET	A	816	47.290	10.260	11.710	1.00	27.50	C
ATOM	4186	CG	MET	A	816	48.410	10.004	12.750	1.00	31.73	C
ATOM	4189	SD	MET	A	816	47.733	9.786	14.449	1.00	35.38	S
ATOM	4190	CE	MET	A	816	47.038	8.358	14.309	1.00	33.86	C
ATOM	4194	C	MET	A	816	46.685	10.684	9.332	1.00	27.46	C
ATOM	4195	O	MET	A	816	46.606	9.769	8.502	1.00	26.95	O
ATOM	4196	N	GLN	A	817	45.791	11.654	9.418	1.00	27.39	N
ATOM	4198	CA	GLN	A	817	44.628	11.668	8.558	1.00	27.92	C
ATOM	4200	CB	GLN	A	817	43.554	12.624	9.076	1.00	27.63	C
ATOM	4203	CG	GLN	A	817	42.950	12.260	10.414	1.00	29.16	C
ATOM	4206	CD	GLN	A	817	42.262	10.929	10.457	1.00	31.41	C
ATOM	4207	OE1	GLN	A	817	41.024	10.843	10.344	1.00	36.06	O
ATOM	4208	NE2	GLN	A	817	43.035	9.881	10.652	1.00	31.67	N
ATOM	4211	C	GLN	A	817	45.011	11.996	7.108	1.00	27.83	C
ATOM	4212	O	GLN	A	817	44.525	11.359	6.165	1.00	27.95	O
ATOM	4213	N	VAL	A	818	45.888	12.958	6.917	1.00	28.09	N
ATOM	4215	CA	VAL	A	818	46.317	13.248	5.562	1.00	28.52	C
ATOM	4217	CB	VAL	A	818	47.221	14.452	5.472	1.00	28.62	C
ATOM	4219	CG1	VAL	A	818	47.834	14.545	4.071	1.00	29.96	C
ATOM	4223	CG2	VAL	A	818	46.447	15.702	5.757	1.00	29.17	C
ATOM	4227	C	VAL	A	818	46.982	11.994	4.926	1.00	28.64	C
ATOM	4228	O	VAL	A	818	46.704	11.661	3.763	1.00	27.81	O
ATOM	4229	N	GLY	A	819	47.793	11.275	5.699	1.00	27.84	N
ATOM	4231	CA	GLY	A	819	48.493	10.127	5.168	1.00	27.87	C
ATOM	4234	C	GLY	A	819	47.525	9.014	4.788	1.00	27.91	C
ATOM	4235	O	GLY	A	819	47.700	8.314	3.792	1.00	27.09	O
ATOM	4236	N	PHE	A	820	46.505	8.848	5.606	1.00	27.82	N
ATOM	4238	CA	PHE	A	820	45.521	7.812	5.419	1.00	28.51	C
ATOM	4240	CB	PHE	A	820	44.730	7.606	6.720	1.00	29.12	C
ATOM	4243	CG	PHE	A	820	43.822	6.403	6.710	1.00	30.74	C
ATOM	4244	CD1	PHE	A	820	44.319	5.143	7.000	1.00	33.05	C
ATOM	4246	CE1	PHE	A	820	43.486	4.028	7.004	1.00	33.70	C
ATOM	4248	CZ	PHE	A	820	42.149	4.170	6.733	1.00	34.30	C
ATOM	4250	CE2	PHE	A	820	41.622	5.438	6.498	1.00	35.70	C
ATOM	4252	CD2	PHE	A	820	42.471	6.547	6.470	1.00	34.48	C
ATOM	4254	C	PHE	A	820	44.627	8.161	4.231	1.00	28.44	C
ATOM	4255	O	PHE	A	820	44.289	7.300	3.437	1.00	29.38	O
ATOM	4256	N	ILE	A	821	44.288	9.425	4.075	1.00	28.63	N
ATOM	4258	CA	ILE	A	821	43.512	9.861	2.914	1.00	29.24	C
ATOM	4260	CB	ILE	A	821	43.138	11.354	3.044	1.00	29.40	C
ATOM	4262	CG1	ILE	A	821	42.064	11.518	4.136	1.00	30.02	C
ATOM	4265	CD1	ILE	A	821	41.748	12.975	4.560	1.00	31.19	C
ATOM	4269	CG2	ILE	A	821	42.656	11.908	1.709	1.00	29.92	C
ATOM	4273	C	ILE	A	821	44.266	9.592	1.618	1.00	29.05	C

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ATOM	4274	O	ILE A 821	43.742	8.990	0.711	1.00	28.50	O
ATOM	4275	N	ASP A 822	45.520	10.008	1.569	1.00	29.41	N
ATOM	4277	CA	ASP A 822	46.356	9.821	0.390	1.00	29.40	C
ATOM	4279	CB	ASP A 822	47.694	10.533	0.583	1.00	30.20	C
ATOM	4282	CG	ASP A 822	47.578	12.026	0.409	1.00	30.26	C
ATOM	4283	OD1	ASP A 822	46.456	12.484	0.103	1.00	33.07	O
ATOM	4284	OD2	ASP A 822	48.535	12.817	0.581	1.00	31.23	O
ATOM	4285	C	ASP A 822	46.604	8.377	0.037	1.00	29.48	C
ATOM	4286	O	ASP A 822	46.465	7.996	-1.110	1.00	30.16	O
ATOM	4287	N	ALA A 823	46.947	7.557	1.021	1.00	29.03	N
ATOM	4289	CA	ALA A 823	47.291	6.173	0.780	1.00	28.80	C
ATOM	4291	CB	ALA A 823	48.068	5.616	1.972	1.00	28.84	C
ATOM	4295	C	ALA A 823	46.087	5.270	0.480	1.00	29.37	C
ATOM	4296	O	ALA A 823	46.188	4.359	-0.334	1.00	28.45	O
ATOM	4297	N	ILE A 824	44.968	5.514	1.157	1.00	30.57	N
ATOM	4299	CA	ILE A 824	43.870	4.549	1.248	1.00	31.06	C
ATOM	4301	CB	ILE A 824	43.657	4.173	2.744	1.00	31.11	C
ATOM	4303	CG1	ILE A 824	44.880	3.451	3.316	1.00	32.33	C
ATOM	4306	CD1	ILE A 824	45.376	2.247	2.543	1.00	33.90	C
ATOM	4310	CG2	ILE A 824	42.377	3.424	2.948	1.00	31.53	C
ATOM	4314	C	ILE A 824	42.546	5.075	0.675	1.00	31.38	C
ATOM	4315	O	ILE A 824	41.849	4.343	-0.013	1.00	31.82	O
ATOM	4316	N	CYS A 825	42.188	6.319	0.977	1.00	31.69	N
ATOM	4318	CA	CYS A 825	40.791	6.784	0.810	1.00	32.07	C
ATOM	4320	CB	CYS A 825	40.430	7.766	1.923	1.00	31.91	C
ATOM	4323	SG	CYS A 825	40.509	7.071	3.573	1.00	33.39	S
ATOM	4324	C	CYS A 825	40.477	7.449	-0.521	1.00	32.49	C
ATOM	4325	O	CYS A 825	39.491	7.110	-1.186	1.00	32.44	O
ATOM	4326	N	LEU A 826	41.305	8.411	-0.887	1.00	32.14	N
ATOM	4328	CA	LEU A 826	41.068	9.228	-2.060	1.00	33.82	C
ATOM	4330	CB	LEU A 826	42.279	10.141	-2.258	1.00	34.35	C
ATOM	4333	CG	LEU A 826	42.094	11.541	-2.771	1.00	36.34	C
ATOM	4335	CD1	LEU A 826	41.169	12.322	-1.846	1.00	37.86	C
ATOM	4339	CD2	LEU A 826	43.498	12.193	-2.926	1.00	36.47	C
ATOM	4343	C	LEU A 826	40.796	8.398	-3.330	1.00	33.83	C
ATOM	4344	O	LEU A 826	39.797	8.594	-4.012	1.00	33.90	O
ATOM	4345	N	GLN A 827	41.676	7.453	-3.629	1.00	33.95	N
ATOM	4347	CA	GLN A 827	41.520	6.625	-4.814	1.00	34.31	C
ATOM	4349	CB	GLN A 827	42.734	5.712	-5.013	1.00	34.21	C
ATOM	4352	CG	GLN A 827	43.454	5.940	-6.309	1.00	37.79	C
ATOM	4355	CD	GLN A 827	44.483	4.845	-6.624	1.00	39.98	C
ATOM	4356	OE1	GLN A 827	45.145	4.330	-5.722	1.00	39.97	O
ATOM	4357	NE2	GLN A 827	44.598	4.492	-7.891	1.00	37.98	N
ATOM	4360	C	GLN A 827	40.237	5.791	-4.799	1.00	33.59	C
ATOM	4361	O	GLN A 827	39.624	5.592	-5.826	1.00	33.68	O
ATOM	4362	N	LEU A 828	39.857	5.276	-3.644	1.00	33.46	N
ATOM	4364	CA	LEU A 828	38.632	4.495	-3.529	1.00	33.05	C
ATOM	4366	CB	LEU A 828	38.542	3.885	-2.143	1.00	33.17	C
ATOM	4369	CG	LEU A 828	37.237	3.166	-1.802	1.00	35.09	C
ATOM	4371	CD1	LEU A 828	36.965	2.045	-2.823	1.00	37.34	C
ATOM	4375	CD2	LEU A 828	37.321	2.625	-0.429	1.00	34.66	C
ATOM	4379	C	LEU A 828	37.356	5.313	-3.849	1.00	32.72	C
ATOM	4380	O	LEU A 828	36.474	4.839	-4.560	1.00	31.03	O
ATOM	4381	N	TYR A 829	37.250	6.521	-3.292	1.00	32.51	N
ATOM	4383	CA	TYR A 829	36.064	7.347	-3.485	1.00	31.96	C
ATOM	4385	CB	TYR A 829	35.979	8.420	-2.386	1.00	31.66	C
ATOM	4388	CG	TYR A 829	35.683	7.843	-1.009	1.00	32.31	C
ATOM	4389	CD1	TYR A 829	36.587	7.999	0.056	1.00	30.90	C
ATOM	4391	CE1	TYR A 829	36.342	7.453	1.282	1.00	32.79	C
ATOM	4393	CZ	TYR A 829	35.174	6.718	1.492	1.00	33.50	C
ATOM	4394	OH	TYR A 829	34.918	6.191	2.724	1.00	29.58	O
ATOM	4396	CE2	TYR A 829	34.259	6.547	0.470	1.00	32.83	C
ATOM	4398	CD2	TYR A 829	34.526	7.101	-0.780	1.00	32.09	C
ATOM	4400	C	TYR A 829	36.061	7.926	-4.926	1.00	32.09	C
ATOM	4401	O	TYR A 829	35.013	8.092	-5.507	1.00	31.90	O
ATOM	4402	N	GLU A 830	37.237	8.178	-5.505	1.00	32.32	N
ATOM	4404	CA	GLU A 830	37.357	8.521	-6.938	1.00	33.53	C

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ATOM   4406  CB   GLU A 830      38.813   8.832  -7.374  1.00 33.92           C
ATOM   4409  CG   GLU A 830      39.422  10.137  -6.848  1.00 36.91           C
ATOM   4412  CD   GLU A 830      40.952  10.292  -7.084  1.00 42.60           C
ATOM   4413  OE1  GLU A 830      41.515  11.357  -6.706  1.00 46.07           O
ATOM   4414  OE2  GLU A 830      41.626   9.381  -7.636  1.00 45.22           O
ATOM   4415  C    GLU A 830      36.817   7.382  -7.788  1.00 33.38           C
ATOM   4416  O    GLU A 830      35.994   7.600  -8.691  1.00 32.83           O
ATOM   4417  N    ALA A 831      37.261   6.165  -7.487  1.00 33.32           N
ATOM   4419  CA   ALA A 831      36.801   4.983  -8.232  1.00 33.85           C
ATOM   4421  CB   ALA A 831      37.571   3.712  -7.831  1.00 33.75           C
ATOM   4425  C    ALA A 831      35.301   4.760  -8.066  1.00 33.80           C
ATOM   4426  O    ALA A 831      34.634   4.435  -9.027  1.00 34.04           O
ATOM   4427  N    LEU A 832      34.778   4.935  -6.856  1.00 33.40           N
ATOM   4429  CA   LEU A 832      33.353   4.752  -6.619  1.00 33.24           C
ATOM   4431  CB   LEU A 832      33.023   4.977  -5.146  1.00 33.06           C
ATOM   4434  CG   LEU A 832      31.587   4.714  -4.691  1.00 33.84           C
ATOM   4436  CD1  LEU A 832      31.165   3.309  -5.086  1.00 33.85           C
ATOM   4440  CD2  LEU A 832      31.420   4.916  -3.160  1.00 36.52           C
ATOM   4444  C    LEU A 832      32.530   5.734  -7.451  1.00 32.81           C
ATOM   4445  O    LEU A 832      31.456   5.410  -7.942  1.00 32.89           O
ATOM   4446  N    THR A 833      33.030   6.952  -7.565  1.00 32.75           N
ATOM   4448  CA   THR A 833      32.333   8.020  -8.272  1.00 32.75           C
ATOM   4450  CB   THR A 833      33.055   9.337  -7.978  1.00 33.07           C
ATOM   4452  OG1  THR A 833      32.827   9.686  -6.599  1.00 33.82           O
ATOM   4454  CG2  THR A 833      32.504  10.472  -8.751  1.00 33.21           C
ATOM   4458  C    THR A 833      32.228   7.728  -9.772  1.00 32.38           C
ATOM   4459  O    THR A 833      31.234   8.077 -10.405  1.00 31.86           O
ATOM   4460  N    HIS A 834      33.233   7.058 -10.321  1.00 32.05           N
ATOM   4462  CA   HIS A 834      33.201   6.605 -11.700  1.00 32.54           C
ATOM   4464  CB   HIS A 834      34.578   6.085 -12.143  1.00 32.85           C
ATOM   4467  CG   HIS A 834      35.565   7.170 -12.448  1.00 34.19           C
ATOM   4468  ND1  HIS A 834      35.416   8.036 -13.515  1.00 36.12           N
ATOM   4470  CE1  HIS A 834      36.436   8.876 -13.540  1.00 36.63           C
ATOM   4472  NE2  HIS A 834      37.243   8.587 -12.532  1.00 36.17           N
ATOM   4474  CD2  HIS A 834      36.725   7.519 -11.838  1.00 35.04           C
ATOM   4476  C    HIS A 834      32.128   5.529 -11.913  1.00 32.37           C
ATOM   4477  O    HIS A 834      31.503   5.484 -12.958  1.00 30.88           O
ATOM   4478  N    VAL A 835      31.921   4.668 -10.922  1.00 32.30           N
ATOM   4480  CA   VAL A 835      30.862   3.658 -11.018  1.00 32.46           C
ATOM   4482  CB   VAL A 835      31.036   2.566  -9.940  1.00 32.60           C
ATOM   4484  CG1  VAL A 835      29.819   1.639  -9.907  1.00 33.06           C
ATOM   4488  CG2  VAL A 835      32.313   1.764 -10.185  1.00 32.37           C
ATOM   4492  C    VAL A 835      29.474   4.293 -10.870  1.00 32.47           C
ATOM   4493  O    VAL A 835      28.527   3.897 -11.548  1.00 32.48           O
ATOM   4494  N    SER A 836      29.359   5.241  -9.935  1.00 32.44           N
ATOM   4496  CA   SER A 836      28.134   5.995  -9.720  1.00 32.19           C
ATOM   4498  CB   SER A 836      27.294   5.378  -8.628  1.00 32.33           C
ATOM   4501  OG   SER A 836      26.015   5.987  -8.624  1.00 34.05           O
ATOM   4503  C    SER A 836      28.453   7.431  -9.366  1.00 31.80           C
ATOM   4504  O    SER A 836      28.979   7.724  -8.286  1.00 31.10           O
ATOM   4505  N    GLU A 837      28.132   8.331 -10.288  1.00 31.31           N
ATOM   4507  CA   GLU A 837      28.449   9.737 -10.127  1.00 31.78           C
ATOM   4509  CB   GLU A 837      28.084  10.510 -11.401  1.00 32.41           C
ATOM   4512  CG   GLU A 837      28.646  11.930 -11.471  1.00 36.07           C
ATOM   4515  CD   GLU A 837      30.173  11.984 -11.392  1.00 41.36           C
ATOM   4516  OE1  GLU A 837      30.848  11.020 -11.858  1.00 43.72           O
ATOM   4517  OE2  GLU A 837      30.700  13.002 -10.869  1.00 45.04           O
ATOM   4518  C    GLU A 837      27.746  10.346  -8.910  1.00 30.90           C
ATOM   4519  O    GLU A 837      28.237  11.318  -8.344  1.00 30.45           O
ATOM   4520  N    ASP A 838      26.615   9.771  -8.509  1.00 30.19           N
ATOM   4522  CA   ASP A 838      25.896  10.213  -7.307  1.00 30.79           C
ATOM   4524  CB   ASP A 838      24.494   9.626  -7.312  1.00 30.45           C
ATOM   4527  CG   ASP A 838      23.733   9.988  -8.566  1.00 33.30           C
ATOM   4528  OD1  ASP A 838      23.271  11.135  -8.642  1.00 30.59           O
ATOM   4529  OD2  ASP A 838      23.594   9.204  -9.539  1.00 37.40           O
ATOM   4530  C    ASP A 838      26.595   9.856  -5.964  1.00 30.92           C
ATOM   4531  O    ASP A 838      26.143  10.267  -4.897  1.00 30.59           O
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ATOM	4532	N	CYS	A	839	27.662	9.074	-6.012	1.00	30.74	N
ATOM	4534	CA	CYS	A	839	28.507	8.897	-4.824	1.00	31.60	C
ATOM	4536	CB	CYS	A	839	29.171	7.532	-4.846	1.00	31.36	C
ATOM	4539	SG	CYS	A	839	28.024	6.171	-4.545	1.00	32.85	S
ATOM	4540	C	CYS	A	839	29.562	10.003	-4.724	1.00	31.85	C
ATOM	4541	O	CYS	A	839	30.388	9.989	-3.819	1.00	31.38	O
ATOM	4542	N	PHE	A	840	29.491	10.986	-5.631	1.00	32.36	N
ATOM	4544	CA	PHE	A	840	30.387	12.136	-5.597	1.00	32.57	C
ATOM	4546	CB	PHE	A	840	29.990	13.214	-6.615	1.00	32.56	C
ATOM	4549	CG	PHE	A	840	30.852	14.450	-6.531	1.00	33.86	C
ATOM	4550,	CD1	PHE	A	840	32.178	14.413	-6.946	1.00	34.98	C
ATOM	4552	CE1	PHE	A	840	32.988	15.529	-6.838	1.00	34.46	C
ATOM	4554	CZ	PHE	A	840	32.480	16.693	-6.291	1.00	34.57	C
ATOM	4556	CE2	PHE	A	840	31.165	16.741	-5.860	1.00	34.02	C
ATOM	4558	CD2	PHE	A	840	30.359	15.627	-5.978	1.00	34.40	C
ATOM	4560	C	PHE	A	840	30.548	12.796	-4.211	1.00	32.63	C
ATOM	4561	O	PHE	A	840	31.668	13.162	-3.875	1.00	33.01	O
ATOM	4562	N	PRO	A	841	29.491	12.988	-3.412	1.00	32.31	N
ATOM	4563	CA	PRO	A	841	29.676	13.675	-2.124	1.00	32.29	C
ATOM	4565	CB	PRO	A	841	28.258	13.741	-1.530	1.00	32.33	C
ATOM	4568	CG	PRO	A	841	27.349	13.598	-2.712	1.00	32.91	C
ATOM	4571	CD	PRO	A	841	28.075	12.639	-3.630	1.00	32.72	C
ATOM	4574	C	PRO	A	841	30.657	12.981	-1.180	1.00	31.80	C
ATOM	4575	O	PRO	A	841	31.259	13.662	-0.373	1.00	32.29	O
ATOM	4576	N	LEU	A	842	30.840	11.675	-1.294	1.00	31.97	N
ATOM	4578	CA	LEU	A	842	31.839	10.973	-0.478	1.00	32.24	C
ATOM	4580	CB	LEU	A	842	31.730	9.457	-0.626	1.00	31.80	C
ATOM	4583	CG	LEU	A	842	30.421	8.832	-0.137	1.00	34.69	C
ATOM	4585	CD1	LEU	A	842	30.347	7.408	-0.600	1.00	37.43	C
ATOM	4589	CD2	LEU	A	842	30.248	8.889	1.388	1.00	36.33	C
ATOM	4593	C	LEU	A	842	33.240	11.442	-0.847	1.00	31.96	C
ATOM	4594	O	LEU	A	842	34.037	11.751	0.023	1.00	32.60	O
ATOM	4595	N	LEU	A	843	33.531	11.497	-2.140	1.00	31.51	N
ATOM	4597	CA	LEU	A	843	34.797	12.014	-2.618	1.00	31.36	C
ATOM	4599	CB	LEU	A	843	34.873	11.858	-4.150	1.00	31.83	C
ATOM	4602	CG	LEU	A	843	36.051	12.457	-4.915	1.00	31.94	C
ATOM	4604	CD1	LEU	A	843	37.368	11.874	-4.389	1.00	32.96	C
ATOM	4608	CD2	LEU	A	843	35.880	12.174	-6.413	1.00	33.16	C
ATOM	4612	C	LEU	A	843	34.992	13.486	-2.243	1.00	30.91	C
ATOM	4613	O	LEU	A	843	36.033	13.876	-1.752	1.00	30.62	O
ATOM	4614	N	ASP	A	844	33.989	14.306	-2.501	1.00	31.32	N
ATOM	4616	CA	ASP	A	844	34.071	15.736	-2.204	1.00	31.76	C
ATOM	4618	CB	ASP	A	844	32.764	16.414	-2.608	1.00	32.08	C
ATOM	4621	CG	ASP	A	844	32.871	17.923	-2.647	1.00	32.63	C
ATOM	4622	OD1	ASP	A	844	33.895	18.463	-3.106	1.00	35.84	O
ATOM	4623	OD2	ASP	A	844	31.970	18.655	-2.232	1.00	33.70	O
ATOM	4624	C	ASP	A	844	34.385	15.959	-0.708	1.00	31.46	C
ATOM	4625	O	ASP	A	844	35.266	16.740	-0.362	1.00	31.17	O
ATOM	4626	N	GLY	A	845	33.694	15.229	0.163	1.00	31.38	N
ATOM	4628	CA	GLY	A	845	33.878	15.355	1.600	1.00	31.48	C
ATOM	4631	C	GLY	A	845	35.266	14.898	2.028	1.00	31.50	C
ATOM	4632	O	GLY	A	845	35.919	15.545	2.852	1.00	31.47	O
ATOM	4633	N	CYS	A	846	35.731	13.790	1.457	1.00	31.20	N
ATOM	4635	CA	CYS	A	846	37.101	13.360	1.667	1.00	30.97	C
ATOM	4637	CB	CYS	A	846	37.340	12.059	0.908	1.00	31.85	C
ATOM	4640	SG	CYS	A	846	38.940	11.316	1.228	1.00	32.79	S
ATOM	4641	C	CYS	A	846	38.132	14.435	1.253	1.00	30.87	C
ATOM	4642	O	CYS	A	846	39.072	14.739	1.992	1.00	29.64	O
ATOM	4643	N	ARG	A	847	37.958	15.003	0.067	1.00	30.89	N
ATOM	4645	CA	ARG	A	847	38.833	16.059	-0.419	1.00	30.94	C
ATOM	4647	CB	ARG	A	847	38.417	16.506	-1.823	1.00	31.78	C
ATOM	4650	CG	ARG	A	847	38.945	15.636	-2.956	1.00	33.28	C
ATOM	4653	CD	ARG	A	847	38.473	16.108	-4.324	1.00	34.97	C
ATOM	4656	NE	ARG	A	847	38.881	15.180	-5.380	1.00	37.42	N
ATOM	4658	CZ	ARG	A	847	38.319	15.114	-6.588	1.00	38.64	C
ATOM	4659	NH1	ARG	A	847	37.290	15.882	-6.915	1.00	37.39	N
ATOM	4662	NH2	ARG	A	847	38.782	14.241	-7.472	1.00	40.75	N

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ATOM    4665  C    ARG A 847      38.838  17.291   0.489  1.00 30.67           C
ATOM    4666  O    ARG A 847      39.898  17.891   0.708  1.00 30.13           O
ATOM    4667  N    LYS A 848      37.669  17.672   1.010  1.00 30.00           N
ATOM    4669  CA   LYS A 848      37.572  18.873   1.840  1.00 30.06           C
ATOM    4671  CB   LYS A 848      36.119  19.316   2.035  1.00 30.40           C
ATOM    4674  CG   LYS A 848      35.493  19.974   0.810  1.00 32.67           C
ATOM    4677  CD   LYS A 848      33.969  20.151   1.034  1.00 35.48           C
ATOM    4680  CE   LYS A 848      33.273  20.910  -0.099  1.00 35.58           C
ATOM    4683  NZ   LYS A 848      31.803  20.591  -0.131  1.00 36.69           N
ATOM    4687  C    LYS A 848      38.244  18.652   3.185  1.00 29.16           C
ATOM    4688  O    LYS A 848      38.875  19.561   3.694  1.00 28.25           O
ATOM    4689  N    ASN A 849      38.131  17.434   3.728  1.00 29.15           N
ATOM    4691  CA   ASN A 849      38.786  17.075   5.002  1.00 29.60           C
ATOM    4693  CB   ASN A 849      38.258  15.748   5.583  1.00 29.71           C
ATOM    4696  CG   ASN A 849      36.826  15.867   6.117  1.00 28.85           C
ATOM    4697  OD1  ASN A 849      36.402  16.925   6.530  1.00 32.39           O
ATOM    4698  ND2  ASN A 849      36.093  14.779   6.097  1.00 28.94           N
ATOM    4701  C    ASN A 849      40.298  17.023   4.862  1.00 29.97           C
ATOM    4702  O    ASN A 849      41.024  17.445   5.777  1.00 30.10           O
ATOM    4703  N    ARG A 850      40.783  16.550   3.710  1.00 30.30           N
ATOM    4705  CA   ARG A 850      42.224  16.548   3.441  1.00 30.06           C
ATOM    4707  CB   ARG A 850      42.525  15.868   2.103  1.00 30.76           C
ATOM    4710  CG   ARG A 850      43.997  15.480   1.922  1.00 30.45           C
ATOM    4713  CD   ARG A 850      44.536  15.663   0.516  1.00 31.23           C
ATOM    4716  NE   ARG A 850      45.953  15.294   0.431  1.00 31.15           N
ATOM    4718  CZ   ARG A 850      46.981  16.122   0.656  1.00 31.68           C
ATOM    4719  NH1  ARG A 850      48.226  15.661   0.572  1.00 31.87           N
ATOM    4722  NH2  ARG A 850      46.796  17.395   0.962  1.00 32.03           N
ATOM    4725  C    ARG A 850      42.765  17.979   3.457  1.00 30.37           C
ATOM    4726  O    ARG A 850      43.801  18.245   4.058  1.00 29.83           O
ATOM    4727  N    GLN A 851      42.045  18.895   2.812  1.00 31.08           N
ATOM    4729  CA   GLN A 851      42.403  20.317   2.806  1.00 32.13           C
ATOM    4731  CB   GLN A 851      41.380  21.178   2.035  1.00 32.54           C
ATOM    4734  CG   GLN A 851      41.601  21.299   0.549  1.00 35.11           C
ATOM    4737  CD   GLN A 851      40.739  22.400  -0.088  1.00 37.85           C
ATOM    4738  OE1  GLN A 851      41.250  23.451  -0.507  1.00 38.21           O
ATOM    4739  NE2  GLN A 851      39.425  22.157  -0.155  1.00 39.86           N
ATOM    4742  C    GLN A 851      42.488  20.837   4.238  1.00 31.69           C
ATOM    4743  O    GLN A 851      43.473  21.471   4.608  1.00 31.56           O
ATOM    4744  N    LYS A 852      41.458  20.548   5.034  1.00 31.11           N
ATOM    4746  CA   LYS A 852      41.385  21.025   6.412  1.00 31.32           C
ATOM    4748  CB   LYS A 852      39.988  20.757   7.010  1.00 31.63           C
ATOM    4751  CG   LYS A 852      38.850  21.660   6.454  1.00 32.95           C
ATOM    4754  CD   LYS A 852      38.794  23.023   7.177  1.00 35.29           C
ATOM    4757  CE   LYS A 852      37.794  24.003   6.545  1.00 36.53           C
ATOM    4760  NZ   LYS A 852      38.394  25.348   6.227  1.00 38.20           N
ATOM    4764  C    LYS A 852      42.509  20.444   7.299  1.00 30.77           C
ATOM    4765  O    LYS A 852      43.193  21.190   8.003  1.00 31.25           O
ATOM    4766  N    TRP A 853      42.732  19.135   7.238  1.00 30.53           N
ATOM    4768  CA   TRP A 853      43.836  18.505   7.981  1.00 30.28           C
ATOM    4770  CB   TRP A 853      43.772  16.973   7.904  1.00 30.25           C
ATOM    4773  CG   TRP A 853      42.685  16.327   8.734  1.00 29.17           C
ATOM    4774  CD1  TRP A 853      41.682  15.521   8.277  1.00 29.72           C
ATOM    4776  NE1  TRP A 853      40.895  15.092   9.318  1.00 27.75           N
ATOM    4778  CE2  TRP A 853      41.394  15.608  10.489  1.00 29.96           C
ATOM    4779  CD2  TRP A 853      42.519  16.395  10.158  1.00 29.49           C
ATOM    4780  CE3  TRP A 853      43.196  17.053  11.192  1.00 29.21           C
ATOM    4782  CZ3  TRP A 853      42.739  16.904  12.502  1.00 30.37           C
ATOM    4784  CH2  TRP A 853      41.628  16.120  12.798  1.00 28.68           C
ATOM    4786  CZ2  TRP A 853      40.930  15.475  11.810  1.00 30.57           C
ATOM    4788  C    TRP A 853      45.213  18.967   7.508  1.00 30.57           C
ATOM    4789  O    TRP A 853      46.093  19.176   8.332  1.00 29.97           O
ATOM    4790  N    GLN A 854      45.405  19.133   6.195  1.00 31.37           N
ATOM    4792  CA   GLN A 854      46.696  19.613   5.660  1.00 32.06           C
ATOM    4794  CB   GLN A 854      46.722  19.603   4.131  1.00 32.60           C
ATOM    4797  CG   GLN A 854      48.091  20.008   3.493  1.00 34.51           C
ATOM    4800  CD   GLN A 854      49.224  19.077   3.896  1.00 35.78           C
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ATOM   4801  OE1 GLN A 854      48.990  17.924   4.265  1.00 35.86       O
ATOM   4802  NE2 GLN A 854      50.450  19.580   3.842  1.00 37.93       N
ATOM   4805  C   GLN A 854      47.025  21.017   6.144  1.00 32.21       C
ATOM   4806  O   GLN A 854      48.164  21.287   6.460  1.00 31.44       O
ATOM   4807  N   ALA A 855      46.019  21.891   6.207  1.00 32.96       N
ATOM   4809  CA  ALA A 855      46.198  23.258   6.710  1.00 33.54       C
ATOM   4811  CB  ALA A 855      44.943  24.112   6.487  1.00 33.09       C
ATOM   4815  C   ALA A 855      46.553  23.222   8.185  1.00 34.08       C
ATOM   4816  O   ALA A 855      47.357  24.019   8.641  1.00 34.67       O
ATOM   4817  N   LEU A 856      45.956  22.291   8.921  1.00 34.47       N
ATOM   4819  CA  LEU A 856      46.308  22.066  10.318  1.00 35.05       C
ATOM   4821  CB  LEU A 856      45.275  21.160  10.984  1.00 35.45       C
ATOM   4824  CG  LEU A 856      44.140  21.879  11.685  1.00 35.64       C
ATOM   4826  CD1 LEU A 856      43.111  20.854  12.128  1.00 35.60       C
ATOM   4830  CD2 LEU A 856      44.669  22.679  12.876  1.00 36.89       C
ATOM   4834  C   LEU A 856      47.713  21.487  10.520  1.00 35.54       C
ATOM   4835  O   LEU A 856      48.405  21.871  11.469  1.00 36.00       O
ATOM   4836  N   ALA A 857      48.151  20.596   9.632  1.00 36.23       N
ATOM   4838  CA  ALA A 857      49.512  20.032   9.707  1.00 36.86       C
ATOM   4840  CB  ALA A 857      49.675  18.886   8.730  1.00 36.47       C
ATOM   4844  C   ALA A 857      50.583  21.096   9.452  1.00 38.02       C
ATOM   4845  O   ALA A 857      51.708  20.990   9.947  1.00 38.57       O
ATOM   4846  N   GLU A 858      50.220  22.120   8.689  1.00 39.06       N
ATOM   4848  CA  GLU A 858      51.147  23.160   8.274  1.00 39.95       C
ATOM   4850  CB  GLU A 858      50.714  23.720   6.921  1.00 40.18       C
ATOM   4853  CG  GLU A 858      51.060  22.815   5.744  1.00 40.91       C
ATOM   4856  CD  GLU A 858      50.697  23.429   4.408  1.00 42.60       C
ATOM   4857  OE1 GLU A 858      50.787  24.669   4.283  1.00 45.70       O
ATOM   4858  OE2 GLU A 858      50.337  22.685   3.475  1.00 43.19       O
ATOM   4859  C   GLU A 858      51.198  24.263   9.317  1.00 40.65       C
ATOM   4860  O   GLU A 858      52.258  24.808   9.616  1.00 40.62       O
ATOM   4861  N   GLN A 859      50.034  24.584   9.870  1.00 41.69       N
ATOM   4863  CA  GLN A 859      49.931  25.557  10.945  1.00 42.43       C
ATOM   4865  CB  GLN A 859      48.473  25.911  11.212  1.00 42.25       C
ATOM   4868  CG  GLN A 859      48.283  27.204  12.027  1.00 42.92       C
ATOM   4871  CD  GLN A 859      47.100  27.140  12.973  1.00 42.72       C
ATOM   4872  OE1 GLN A 859      46.619  26.054  13.304  1.00 42.44       O
ATOM   4873  NE2 GLN A 859      46.620  28.307  13.404  1.00 42.81       N
ATOM   4876  C   GLN A 859      50.582  25.026  12.218  1.00 43.48       C
ATOM   4877  O   GLN A 859      51.049  25.817  13.037  1.00 44.43       O
ATOM   4878  N   GLN A 860      50.640  23.702  12.374  1.00 44.48       N
ATOM   4880  CA  GLN A 860      51.220  23.091  13.571  1.00 45.14       C
ATOM   4882  CB  GLN A 860      50.674  21.680  13.810  1.00 45.53       C
ATOM   4885  CG  GLN A 860      49.391  21.646  14.675  1.00 45.81       C
ATOM   4888  CD  GLN A 860      48.904  20.223  14.929  1.00 48.17       C
ATOM   4889  OE1 GLN A 860      47.774  20.011  15.408  1.00 49.37       O
ATOM   4890  NE2 GLN A 860      49.747  19.239  14.593  1.00 48.49       N
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ATOM   4899  CB  GLU A 861      55.145  22.865  10.637  1.00 46.94       C
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ATOM   4912  CA  LYS A 862      54.433  26.943  13.075  1.00 47.01       C
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ATOM   4923  CE  LYS A 862      56.457  23.740  15.812  1.00 46.64       C
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ATOM   4930  C   LYS A 862      55.078  27.670  11.893  1.00 47.28       C
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ATOM   4935 C6    CIT L  101    48.308   0.359  -4.647  0.50 72.51           C
ATOM   4936 O6    CIT L  101    47.451  -0.244  -3.887  1.00 72.89           O
ATOM   4938 C3    CIT L  101    48.403  -0.153  -6.141  0.50 73.65           C
ATOM   4939 O7    CIT L  101    47.133  -0.911  -6.214  1.00 72.11           O
ATOM   4941 C4    CIT L  101    48.459   0.972  -7.359  1.00 77.19           C
ATOM   4944 C5    CIT L  101    47.271   1.292  -8.386  1.00 79.59           C
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ATOM   4951 C1    CIT L  101    49.294  -2.684  -6.267  1.00 74.60           C
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ATOM   4964 O     HOH W    4     49.048   8.267   7.910  1.00 45.59           O
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ATOM   4985 O     HOH W   11     16.672  10.547   4.776  1.00 58.12           O
ATOM   4988 O     HOH W   12     41.666  -3.613   8.704  1.00 43.31           O
ATOM   4991 O     HOH W   13     50.372  16.058  12.308  1.00 55.26           O
ATOM   4994 O     HOH W   14     38.665  -2.779   3.708  1.00 39.85           O
ATOM   4997 O     HOH W   15     45.675 -12.816   6.127  1.00 53.14           O
ATOM   5000 O     HOH W   16     34.796   5.945  18.234  1.00 65.49           O
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ATOM   5048 O     HOH W   32     41.988  18.107  -0.911  1.00 62.92           O
ATOM   5051 O     HOH W   33     50.668  16.518   5.962  1.00 76.18           O
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ATOM   5072 O     HOH W   40     26.485  -2.035  22.374  1.00 59.06           O
ATOM   5075 O     HOH W   41     13.524 -16.426  14.869  1.00 56.13           O
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ATOM   5081 O     HOH W   43     44.000   3.616  30.031  1.00 79.86           O
ATOM   5084 O     HOH W   44     29.770 -15.325  -3.436  1.00 72.37           O
ATOM   5087 O     HOH W   45     55.046  -7.275  10.420  1.00 78.57           O
ATOM   5090 O     HOH W   46     42.174  23.377   8.895  1.00 59.81           O
ATOM   5093 O     HOH W   47     45.846   5.171  -3.429  1.00 62.43           O
ATOM   5096 O     HOH W   48     10.721   2.483  -0.428  1.00 71.72           O
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ATOM   5105 O     HOH W   51     37.981  -1.356   6.040  1.00 52.06           O
ATOM   5108 O     HOH W   52     38.803  -5.419  -9.275  1.00 74.64           O
ATOM   5111 O     HOH W   53     41.112  23.777   3.772  1.00 74.75           O
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ATOM   5117   O   HOH  W   55    25.576   -9.292  -16.079   1.00 78.39          O
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ATOM   5126   O   HOH  W   58    48.193    0.023  -15.539   0.50 75.94          O
ATOM   5129   O   HOH  W   59    15.570  -23.396   -5.422   1.00 64.14          O
ATOM   5132   O   HOH  W   60    44.168   -8.356   -7.513   1.00 64.26          O
ATOM   5135   O   HOH  W   61    28.988   -3.170  -14.959   1.00 77.62          O
ATOM   5138   O   HOH  W   62    20.901  -25.326   -2.234   1.00 67.25          O
ATOM   5141   O   HOH  W   63    33.982   16.977    4.436   1.00 59.29          O
ATOM   5144   O   HOH  W   64    37.652   17.395   18.730   1.00 70.50          O
ATOM   5147   O   HOH  W   65    48.714   -1.176   12.509   1.00 55.83          O
ATOM   5150   O   HOH  W   66    23.648   13.228   15.636   1.00 61.83          O
ATOM   5153   O   HOH  W   67    37.930   -5.485   14.629   1.00 62.75          O
ATOM   5156   O   HOH  W   68    39.361    8.113   12.281   1.00 65.09          O
ATOM   5159   O   HOH  W   69    44.416   19.041    0.025   1.00 65.31          O
ATOM   5162   O   HOH  W   70    31.024  -11.670   12.913   1.00 51.04          O
ATOM   5165   O   HOH  W   71    30.803  -10.927   -2.260   1.00 70.95          O
ATOM   5168   O   HOH  W   72    18.394    4.353   24.131   1.00 56.96          O
ATOM   5171   O   HOH  W   73    40.724   -9.915   -6.592   1.00 71.31          O
ATOM   5174   O   HOH  W   74    39.415  -11.451   -1.251   1.00 59.36          O
ATOM   5177   O   HOH  W   75    10.729    6.380    4.422   1.00 64.07          O
ATOM   5180   O   HOH  W   76    32.027  -16.657    1.416   1.00 71.19          O
ATOM   5183   O   HOH  W   77    26.786  -20.606    2.339   1.00 57.23          O
ATOM   5186   O   HOH  W   78    18.583   16.228    4.823   1.00 73.42          O
ATOM   5189   O   HOH  W   79    27.374    7.398    8.872   1.00 62.50          O
ATOM   5192   O   HOH  W   80    35.193  -16.175   10.352   1.00 73.93          O
ATOM   5195   O   HOH  W   81    52.965   18.374    4.826   1.00 72.09          O
ATOM   5198   O   HOH  W   82    11.094    0.000    0.113   1.00 85.89          O
ATOM   5201   O   HOH  W   83    22.715   12.451    0.236   1.00 66.47          O
ATOM   5204   O   HOH  W   84    29.996    6.584    8.368   1.00 57.82          O
ATOM   5207   O   HOH  W   85    42.337   13.541   20.658   1.00 71.89          O
ATOM   5210   O   HOH  W   86    37.358   -4.176   21.021   1.00 72.66          O
ATOM   5213   O   HOH  W   87    13.285   -6.976   -6.448   1.00 67.69          O
ATOM   5216   O   HOH  W   88    43.834   23.530   -1.284   1.00 77.37          O
ATOM   5219   O   HOH  W   89    45.114   22.881    3.087   1.00 68.89          O
ATOM   5222   O   HOH  W   90    44.237  -15.595    6.458   1.00 87.26          O
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Table 2

PCR from Human Kidney QUICK-Clone cDNA (Clontech, #7112-1)

Protein in pET15S: 366 aa   Mass: 42049.2 pI: 6.68


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  1 MGSSHHHHHH SSGLVPRGSH MSAAEEETRE LQSLAAAVVP SAQTLKITDF SFSDFELSDL
 61 ETALCTIRMF TDLNLVQNFQ MKHEVLCRWI LSVKKNYRKN VAYHNWRHAF NTAQCMFAAL
121 KAGKIQNKLT DLEILALLIA ALSHDLDHRG VNNSYIQRSE HPLAQLYCHS IMEHHHFDQC
181 LMILNSPGNQ ILSGLSIEEY KTTLKIIKQA ILATDLALYI KRRGEFFELI RKNQFNLEDP
241 HQKELFLAML MTACDLSAIT KPWPIQQRIA ELVATEFFDQ GDRERKELNI EPTDLMNREK
301 KNKIPSMQVG FIDAICLQLY EALTHVSEDC FPLLDGCRKN RQKWQALAEQ QEKMLINGES
361 GQAKRN
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PDE5A-S: 5'-GTCGTAT CATATG TCAGCAGCAGAGGAAGAAAC-3' 33 mer

PDE5A-A: 5'-TCTGCA GTCGAC AGGCCACTCAGTTCCGCTTG-3' 32 mer

pET15S sequence (PCR product; 1070 bp)

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1981 taaaattact gactttagct tcagtgactt tgagctgtct gatctggaaa cagcactgtg
2041 tacaattcgg atgtttactg acctcaacct tgtgcagaac ttccagatga aacatgaggt
2101 tctttgcaga tggattttaa gtgttaagaa gaattatcgg aagaatgttg cctatcataa
2161 ttggagacat gcctttaata cagctcagtg catgtttgct gctctaaaag caggcaaaat
2221 tcagaacaag ctgactgacc tggagatact tgcattgctg attgctgcac taagccacga
2281 tttggatcac cgtggtgtga ataactctta catcagcga agtgaacatc cacttgccca
2341 gctttactgc cattcaatca tggaacacca tcattttgac cagtgcctga tgattcttaa
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2701 aaaagaactc aacatagaac ccactgatct aatgaacagg gagaagaaaa acaaaatccc
2761 aagtatgcaa gttgggttca tagatgccat ctgcttgcaa ctgtatgagg ccctgacCCA
2821 cgtgtcagag gactgtttcc ctttgctaga tggctgcaga aagaacaggc agaaatggca
2881 ggcccttgca gaacagcagg agaagatgct gattaatggg gaaagcggcc aggccaagcg
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GTCGACTAGAGCCTGCAGTCTCGACCATCATCATCATCATCATTAATAAAAGGGCGAATTCCAGCACACT
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Table 3

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Table 4

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LOCUS           PDE5A                    3106 bp    mRNA      linear    PRI 05-NOV-2002
DEFINITION      Homo sapiens phosphodiesterase 5A, cGMP-specific (PDE5A),
                transcript variant 1, mRNA.
ACCESSION       NM_001083
VERSION         NM_001083.2  GI:15812210
KEYWORDS        .
SOURCE          Homo sapiens (human)
  ORGANISM      Homo sapiens
                Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
                Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE       1  (bases 1 to 3106)
  AUTHORS       Stacey,P., Rulten,S., Dapling,A. and Phillips,S.C.
  TITLE         Molecular cloning and expression of human cGMP-binding
                cGMP-specific phosphodiesterase (PDE5)
  JOURNAL       Biochem. Biophys. Res. Commun. 247 (2), 249-254 (1998)
  MEDLINE       98308101
   PUBMED       9642111
REFERENCE       2  (bases 1 to 3106)
  AUTHORS       Yanaka,N., Kotera,J., Ohtsuka,A., Akatsuka,H., Imai,Y.,
                Michibata,H., Fujishige,K., Kawai,E., Takebayashi,S., Okumura,K.
                and Omori,K.
  TITLE         Expression, structure and chromosomal localization of the human
                cGMP-binding cGMP-specific phosphodiesterase PDE5A gene
  JOURNAL       Eur. J. Biochem. 255 (2), 391-399 (1998)
  MEDLINE       98380237
   PUBMED       9716380
REFERENCE       3  (bases 1 to 3106)
  AUTHORS       Loughney,K., Hill,T.R., Florio,V.A., Uher,L., Rosman,G.J.,
                Wolda,S.L., Jones,B.A., Howard,M.L., McAllister-Lucas,L.M.,
                Sonnenburg,W.K., Francis,S.H., Corbin,J.D., Beavo,J.A. and
                Ferguson,K.
  TITLE         Isolation and characterization of cDNAs encoding PDE5A, a human
                cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide
                phosphodiesterase
  JOURNAL       Gene 216 (1), 139-147 (1998)
  MEDLINE       98382582
   PUBMED       9714779
REFERENCE       4  (bases 1 to 3106)
  AUTHORS       Kotera,J., Fujishige,K., Imai,Y., Kawai,E., Michibata,H.,
                Akatsuka,H., Yanaka,N. and Omori,K.
  TITLE         Genomic origin and transcriptional regulation of two variants of
                cGMP-binding cGMP-specific phosphodiesterases
  JOURNAL       Eur. J. Biochem. 262 (3), 866-873 (1999)
  MEDLINE       99339957
   PUBMED       10411650
REFERENCE       5  (bases 1 to 3106)
  AUTHORS       Lin,C.S., Lau,A., Tu,R. and Lue,T.F.
  TITLE         Identification of three alternative first exons and an intronic
                promoter of human PDE5A gene
  JOURNAL       Biochem. Biophys. Res. Commun. 268 (2), 596-602 (2000)
  MEDLINE       20145478
   PUBMED       10679249
REFERENCE       6  (bases 1 to 3106)
  AUTHORS       Lin,C.S., Lau,A., Tu,R. and Lue,T.F.
  TITLE         Expression of three isoforms of cGMP-binding cGMP-specific
                phosphodiesterase (PDE5) in human penile cavernosum
  JOURNAL       Biochem. Biophys. Res. Commun. 268 (2), 628-635 (2000)
  MEDLINE       20145484
   PUBMED       10679255
REFERENCE       7  (bases 1 to 3106)
  AUTHORS       Lin,C.S., Chow,S., Lau,A., Tu,R. and Lue,T.F.
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TITLE       Identification and regulation of human PDE5A gene promoter
JOURNAL     Biochem. Biophys. Res. Commun. 280 (3), 684-692 (2001)
MEDLINE     21092663
 PUBMED     11162575
COMMENT     REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence was derived from AF043731.1.
            On Oct 1, 2001 this sequence version replaced gi:4505666.
            Summary: This gene encodes a cGMP-binding, cGMP-specific
            phosphodiesterase, a member of the cyclic nucleotide
            phosphodiesterase family. This phosphodiesterase specifically
            hydrolyzes cGMP to 5'-GMP. It is involved in the regulation of
            intracellular concentrations of cyclic nucleotides and is important
            for smooth muscle relaxation in the cardiovascular system.
            Alternative splicing of this gene results in four transcript
            variants encoding distinct isoforms.
            Transcript Variant: This variant (1) encodes the longest isoform
            (1) of this protein.
FEATURES             Location/Qualifiers
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                          /allele="A"
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